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13. ABSTRACT ***(Maximum 200 Words)***

Recently we identified a novel vitamin D analog, 1α-hydroxy-24 ethyl vitamin D_5 (1α(OH)D_5) that showed potent growth inhibitory and cell-differentiating actions in breast cancer cells. Based on our findings in in vitro and in vivo experimental model systems, we hypothesized that 1α(OH)D_5, when administered to women with breast cancer, will induce differentiation of dedifferentiated cells and thereby prevent progression of malignancy. In 1999-2000, we completed the preclinical study in rats. Results showed that 1α(OH)D_5 has no serious toxicity; a hypercalcemic effect was observed at high dose, which was reversible. In vitro study in tissues obtained from patients show that 1α(OH)D_5 has no effect on normal breast epithelial cells, but it induces apoptosis in breast cancer. It also showed apoptotic effect in fibroadenomas. We completed 5 steps in the synthesis of 1α(OH)D_5 for preparation of 1α(OH)D_5 for phase I clinical study. In 2000-2001, we completed preclinical toxicity studies in dogs under GMP. We have completed synthesis of 1α(OH)D_5 under GMP for future clinical trial. In vitro studies in clinical specimens obtained from women suggest that 1α (OH)D_5 has no effect on normal breast tissues; it inhibits cell proliferation in tumor cells. 1α(OH)D_5 or its active metabolite possibly interacts with estrogen receptor. We will be submitting our IND application to the FDA.

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Table of Contents

Introduction

Vitamin D and its analogs have shown potential chemopreventive and chemotherapeutic effects on various malignant tumors (1-14). The active metabolite of vitamin D3, 1,25(OH)2D3, has been shown conclusively to induce differentiation in vitro in a variety of cancer cells, including breast cancer cells (12-14). 1,25(OH)2D3 is hypercalcemic, and thus its use as a preventive and therapeutic agent is limited . Although a number of vitamin D analogs are synthesized, only limited vitamin D-related compounds have reached clinical trial. Recently, we identified a vitamin D analog that showed potent growth inhibitory and cell-differentiating action in breast cancer cells. The effects of $1\alpha(OH)D_5$ were extensively investigated in vitro and in vivo. We aim to pilot $1\alpha(OH)D_5$ from an experimental laboratory model to the clinical setting. The effects of $1\alpha(OH)D_5$ were investigated extensively in in vitro and in vivo experimental models, and the results are summarized below.

- $1\alpha(OH)D_5$ has chemopreventive action in mouse organ culture model (15).
- $1\alpha(OH)D_5$ has chemopreventive action on DMBA-induced mammary tumors in rats (16).
- $1\alpha(OH)D_5$ has both growth inhibitory and cell-differentiating actions in human breast carcinoma cells (17,18).
- $1\alpha(OH)D_5$ supplemented in the diet inhibits the in vivo growth of human breast carcinoma transplanted in athymic mice (18).
- $1\alpha(OH)D_5$ is metabolized into two major metabolites (1,24 and 1,25 vitamin D5) in human breast tumors and nonmalignant breast tissues.
- During the last fiscal year, we have completed preclinical toxicity studies in male and female rats under GLP. Male and female rats were given 1-10 μg/kg body weight $1\alpha(OH)D_5$ by gavage for 28 consecutive days. $1\alpha(OH)D_5$ showed no serious toxic effect. No animals died during the course of study, and no adverse treatment-related clinical signs of toxicity were observed. Increased serum calcium levels were observed in both sexes at the high dose level and in females at mid-dose levels. Microscopic lesions consisting primarily of increased renal mineralization were seen in males at mid- and high-dose levels, and in females at all doses (19).
- The effect of $1\alpha(OH)D_5$ was reversible. Within two weeks after discontinuation of the treatment, serum calcium levels and renal mineralization lesions reached the same levels as the control group (19).
- Under the current contract, during the last funding year, we studied the in vitro effect of $1\alpha(OH)D_5$ on malignant and nonmalignant tissues obtained from breast cancer patients at the time of surgery. $1\alpha(OH)D_5$ had no effect on cell proliferation, cell death, or differentiation markers (casein) in nonmalignant breast tissues (epithelial cells). $1\alpha(OH)D_5$ induced cell death in fibroadenomas. In malignant tumors, 1a(OH)D5 induced apoptosis. (20).

Hypothesis proposed

We hypothesize that (1) $1\alpha(OH)D_5$ administered to women with breast cancer will induce differentiation of dedifferentiated malignant cells and thereby prevent progression of malignancy, and (2) in women with premalignant lesions, $1\alpha(OH)D_5$ will prevent dedifferentiation and thus prevent induction and/or development of breast cancer.

Technical Objectives proposed

The specific objectives of the proposed study are to:

1. Establish and evaluate biomarkers predicting $1\alpha(OH)D_5$ response in malignant breast cancer and DCIS (Ductal Carcinoma in Situ).
2. Study the molecular mechanism by which $1\alpha(OH)D_5$ induces differentiation/inhibits proliferation of breast cancer cells.
3. Perform (according to FDA requirement) preclinical toxicity and pharmacokinetic studies of $1\alpha(OH)D_5$.
4. Initiate a phase I/II trial in advanced breast cancer patients. (During this trial, we will also obtain data on the metabolism of $1\alpha(OH)D_5$ in humans.)

Successful completion of the proposed study will identify a new chemotherapeutic and possibly chemo-preventive agent in breast cancer.

Statement of work and time schedule proposed for 2000-2001

Statement of Work

1. Continue to evaluate biomarkers predicting $1\alpha(OH)D_5$ response in malignant breast cancer and DCIS (Ductal Carcinoma in Situ).
2. Study the molecular mechanism by which $1\alpha(OH)D_5$ induces differentiation/inhibits proliferation of breast cancer cells.
3. Complete (according to FDA requirement) preclinical toxicity and pharmacokinetic studies of $1\alpha(OH)D_5$ in dogs.

Time schedule proposed for the current grant period.

13-17 months: Conduct efficacy studies in athymic mice and determine vitamin D_5 metabolism in the tissues. Evaluate cell surface markers and their alterations by the test agent. Examine binding of D metabolites with estrogen receptors. Transfect ER in the ER- cells, and evaluate the effects of D_5 on the growth parameters of ER- cells stably transfected with ER. Continue studying cell cycle checkpoints in response to $1\alpha(OH)D_5$. Complete toxicity studies under GLP regulation and establish toxicity of $1\alpha(OH)D_5$. Initiate patient enrollment for the Phase I trial with the compound.

18-24 months: Continue studies described in Specific Aims 1 and 2, including efficacy studies in athymic mice, differentiation parameters, transfection of VDR in VDR- ER- MDA-MB cells, and determine the effects of $1\alpha(OH)D_5$ on induction of differentiation as it relates to VDR. Continue with the clinical trial and accrue eligible patients. Examine toxicity and monitor patients throughout the rest of the study period and until the trial is completed.

Results

Is the cell-differentiating effect due to its interaction with ER?

In order to achieve this goal, we have established four different cell lines, as originally proposed in the application. MDA-MB-231 cells were used in this study. In the previous report, we showed that MDA-MB-231 cells show undetectable VDR expression. In vitro, MDA-MB-231 cells fail to show growth-inhibitory response to $1\alpha(OH)D_5$. MDA-MB-231 cells transfected with full-length cDNA for human estrogen receptor were obtained from Dr. Craig Jordan of Northwestern University.

All cell lines were transfected using Lipofectin.

We have generated the following cell lines:

1. MDA-MB-231 transfected with plasmid DNA containing ampicillin-resistance gene and full-length human VDR cDNA.
2. MDA-MB-231 transfected with plasmid containing ampicillin-resistance gene only.
3. MDA-MB-231 (ER cDNA-transfected S-30) cells transfected with plasmid containing zymocin-resistance gene and full-length human VDR cDNA.
4. MDA-MB-231 (S-30) cells transfected with plasmid containing zymocin resistance gene only.

We have confirmed that VDR cDNA-transfected cell lines express VDR (Figures 1 and 2).

$1\alpha(OH)D_5$ inhibits ER expression in S-30 (ER+) VDR-transfected cells.

In order to determine the effect of $1\alpha(OH)D_5$ on ER status, we examined ER expression immunohistochemically in S-30 cells transfected with VDR. As shown in Figure 3, $1\alpha(OH)D_5$ treatment inhibited expression of ER in VDR-transfected S-30 cells. These results indicated that $1\alpha(OH)D_5$ or its metabolite(s) have estrogen receptor-mediated antiestrogenic effect in breast cancer cells.

All cell lines are currently growing in culture, and we are evaluating the effect of $1\alpha(OH)D_5$ on the growth and differentiation of these cells.

Effect of $1\alpha(OH)D_5$ on expression of various genes in BT-474 cells: $1\alpha(OH)D_5$ down-regulates estrogen inducible genes.

In order to determine whether $1\alpha(OH)D_5$ or its metabolites interact with estrogen receptor and probably act as an antiestrogen, we analyzed changes in various genes in control vehicle-treated and $1\alpha(OH)D_5$-treated BT-474 cells. BT-474 cells are estrogen receptor-positive and vitamin D receptor-positive. $1\alpha(OH)D_5$ inhibits both in vivo and in vitro growth of BT-474 cells. Cells were incubated with $1\alpha(OH)D_5$ or vehicle only for four days; RNA was extracted and then subjected to microarray analysis. Table 1 lists the genes which are down-regulated significantly ($p< 0.01$) in $1\alpha(OH)D_5$-treated cells as compared to vehicle treated cells. Many of these genes are regulated by estrogen or progesterone.

Table 1

Gene name	Ratio between treated and control	Comment	References
PS2	5.7	Estrogen-inducible gene	21-30
Progesterone receptor	3.2	Estrogen-inducible gene	31-32
IGFBP-5	3.2	Estrogen-regulated	33-36
IGFBP-4	2.6	-	
Integrin alpha6	1.5	Progesterone receptor-regulated	37
Laminin receptor	1.9		-
Annexin 1	1.7	Glucocorticoid receptor-regulated protein	38

Table 2 shows a list of genes that are up-regulated in $1\alpha(OH)D_5$-treated cells.

Table 2

Name of gene	Ratio of gene expression	Comment	References
Caspase 3	1.7	Enzyme associated with apoptosis, vitamin D action	39
Alpha integrin-binding protein	1.8		-
Calcineurin-binding protein	1.8	Vitamin D-related protein	-
Nucleoporin	1.9		-
Mitochondrial thymidine kinase	1.9		-
Phospholipase C	2.0		-
Cadherin 18	3.5	Differentiation-associated protein	
PKC theta	4.6		40
Vitamin D hydroxylase	6.3	Vitamin-metabolizing enzyme	41

We further confirmed the antiestrogenic property of $1\alpha(OH)D_5$ by examining progesterone receptor protein in BT-474 cells. Our results clearly suggest that $1\alpha(OH)D_5$ inhibits the expression of progesterone receptor in BT-474 cells (data not shown).

Does $1\alpha(OH)D_5$ mediate its action through interaction with VDR?

We have examined competitive binding of $1\alpha(OH)D_5$ with $1,25(OH)_2D_3$ to pure human VDR. For determining binding of $1\alpha(OH)D_5$ to VDR, VDR ligand binding domain (VDR LBD, 20 ng/tube) was incubated with 3H-1,25(OH)2D3 (S.A. 20 mCi/mmol), rat liver nuclear extract (10 mg/tube) in the presence or absence of increasing concentrations of $1\alpha(OH)D_5$ or 1,25(OH)D3 (non-radioactive) at 4°C for 15 hrs. Following incubation, free radioactivity was removed using Dextran-coated charcoal. The samples were mixed with charcoal suspension and incubated at 4°C for 20 min. The samples were centrifuged at 1200 x g for 15 min. Supernatant was mixed with scintillation fluid and radioactivity was determined using a scintillation counter. Percent of binding in the presence of unlabelled ligand was calculated as binding in the presence of unlabeled

ligand divided by total binding in the absence of unlabelled ligand x 100. Our results show that $1\alpha(OH)D_5$ has 1000-fold less binding affinity for VDR than $1,25(OH)_2D_3$ (Figure 4.). These results further suggest that a metabolite of $1\alpha(OH)D_5$ is possibly responsible for the growth-inhibitory and cell-differentiating action.

At present, we are studying the metabolism of $1\alpha(OH)D_5$. Dr. Reddy from Brown University is looking into epimerization of $1\alpha(OH)D_5$ as an active metabolite of $1\alpha(OH)D_5$.

The effect of $1\alpha(OH)D_5$ on various differentiation and proliferation markers in malignant and non-malignant breast tissues obtained from women with confirmed diagnosis of breast cancer.

As noted in the last reporting period, our institution was placed on clinical hold by the NIH. As a result, all of our clinical protocols were also put on hold; thus, we were unable to procure any tissues. However, the hold on the UIC IRB has been lifted, and our protocol has been considered for full review and approved by the UIC IRB committee. Since the IRB approval, we have obtained 20 additional tumors and normal breast tissues. Tissues were incubated with $1\alpha(OH)D_5$ (0.1, 1.0 μM) or vehicle only for 48 hrs. at 37°C. Following incubation, tissues were fixed in formalin and then processed for histopathology. Only those tissues that showed epithelial cell components were further processed for the immunohistochemical studies of Ki-67, VDR, and B-casein. ER and PR contents were examined immunohistochemically only in the original tumor specimens.

As indicated in the last report, we observed that $1\alpha(OH)D_5$ treatment for 48 hrs inhibited the Ki-67 staining (nuclear) in some breast cancers. In tumor tissue treated with $1\alpha(OH)D_5$, a decrease in nuclear staining for Ki-67 was observed. Similarly, it also increased casein expression in selected tumors. Normal nonmalignant breast tissues had no effect on the Ki-67, VDR, or casein expression in the epithelial cells.

We studied alpha2 expression in breast tumors and breast tissues using various antibodies reported to detect alpha2 expression in formalin-fixed paraffin sections of tissues. We used different antigen retrieval agents as suggested by antibody suppliers; however, no consistent results were observed in the alpha2 expression in the tissues studied. We assayed alpha2 integrin expression in frozen tumor sections; however, the number of tissues studied is too small to derive meaning full conclusion.

The following experiments, currently in progress in our laboratory, were proposed in the original application.

1. Study the direct interaction of $1\alpha(OH)D_5$ with estrogen receptor.

2. Identify the active metabolite of $1\alpha(OH)D_5$.

3. Determine in vivo $1\alpha(OH)D_5$'s efficacy at inhibiting the growth of human breast tumors transplanted in athymic mice.

Synthesis of $1\alpha(OH)D_5$ under GMP for future Phase I clinical trial.

During the last funding period, we received 1 gm of $1\alpha(OH)D_5$ for preclinical toxicity study. Dr. Moriarty and his group have prepared 350 mg of $1\alpha(OH)D_5$ for the phase I clinical trial under GMP (see Appendix 2). Additional compound will be prepared in the next six months.

Preclinical Toxicity studies Under GLP.

The preclinical toxicity studies using two species under GLP conditions were proposed in the original application. We completed preclinical toxicity studies in rats, and details were submitted in the last progress report. Four-week toxicity in the rats suggested that $1\alpha(OH)D_5$ administered by gavage for 28 days to adult males and females was well tolerated in rats. At the doses tested, minimal toxic effect was observed in male and female rats.

Preclinical toxicity studies in Dogs.

A 28-day oral toxicity study was conducted in male and female beagle dogs to evaluate the toxicity of $1\alpha(OH)D_5$ administered by gavage for four weeks. $1\alpha(OH)D_5$ was dissolved in ethanol and then further diluted in corn oil. Four different doses were given (5, 10, 30, and 90 μg/kg body weight). Control group received vehicle at equal volume. The study design originally included 6 animals (3 male, 3 female) in lower doses (10, 30 μg) and 10 animals (5 of each sex) in higher doses.

As we observed mortality (2 dogs) in the high-dose (90 μg) group within a week of initiating treatment, the treatment dose in the remaining animals was reduced to 45 μg/kg body weight for the next 3 weeks.

Toxicological endpoints included physical examinations/clinical observations, ophthalmologic examination, body weights, food consumption, clinical pathology (hematology, clinical chemistry, urine analysis), organ weights, and electrocardiographic evaluations. Tissues from all dogs in the vehicle-treated, 10 μg dose, and 30 μg dose groups which were sacrificed were evaluated histopathologically. In addition, target tissues and gross lesions from dogs treated with the 5 μg dose were also evaluated histopathologically.

Administration of $1\alpha(OH)D_5$ at dose levels greater than 5 μg/kg induced symptoms of hypervitaminosis. Eight dogs died or were sacrificed during the study (2 females at 90 μg/kg dose, 3 males at 45 μg/kg dose, and 2 males and a female at 30 μg/kg dose).

Mean body weight and body weight gains were statistically decreased in dogs treated with >5 μg/kg dose by day 8. Body weight loss was 25-43% of their initial body weight. Body weight losses were accompanied by decreased food consumption. Erythrocyte count, hematocrit, and hemoglobin levels increased in both sexes at doses of 10 μg/kg body weight and above, which most likely resulted due to the dehydrated condition of the animals. Serum calcium levels were increased and serum inorganic phosphorus levels were significantly decreased in a dose-dependent manner in both sexes at all dose levels. In addition, females receiving 30 μg or higher doses had decreased alkaline phosphatase, along with increased blood urea nitrogen, cholesterol, and triglyceride levels.

At any dose, no treatment-related ophthalmologic or electrocardiographic changes were observed. At 5 and 10 μg doses of $1\alpha(OH)D_5$, we observed mineralization in the arteries of the spleen (females only) and heart (males only), bone marrow depletion, and cartilage hypoplasia in the femur.

In conclusion, administration of $1\alpha(OH)D_5$ at dose levels 5-90 μg/kg body weight via oral gavage daily for 28 days induced signs of hypervitaminosis. A "no observable effect level" (NOEL) was not established in this study (a detailed report is attached in the appendix).

Plan for the Clinical Trial

The two species preclinical toxicity studies have been completed (see pages 8-9 and Appendix 3). Based on these studies and approval of the protocol and informed consent form (both in English and Spanish) of the Phase 1/2 clinical trial by the U.S. Army Human Research Regulatory Compliance and Quality Review Committee (HSRRB) (Ms. Catherine A. Smith, Human Subjects Protection Specialist), we will submit the amended FDA application (IND #56509) to obtain approval for this clinical trial. Currently, Lutheran General Hospital (LGH) Institutional Review Board (IRB) has approved the same protocol and consent form as has been approved by the US Army HSRRB. However, the UIC IRB (E. Gislason, Ph.D., Vice-Chancellor) is currently withholding approval of these documents pending an internal review. Although the review process is moving along expeditiously, it is possible that this will not be completed by the time the FDA-approved IND is received. Therefore, if approved by the US Army HSRRB, the trial can be initiated at Lutheran General Hospital (LGH). Dr. Jacob Bitran and LGH are included in the original application with appropriate funding to

proceed with patient accrual at their location. When the clinical hold is lifted by the UIC IRB and the protocol and consent form have been agreed upon, subjects can be enrolled at UIH as well. However, most subjects will likely be enrolled from LGH since Dr. Bitran and his group see more breast cancer patients than do the group at UIH.

Key Research Accomplishments during the current funding year

1. We have completed preclinical toxicity studies in dogs under GMP. $1\alpha(OH)D_5$ was tested (5-45/90 μg per kg body weight dose). The compound was given to animals daily by gavage for 28 days. At 5 μg/kg body weight dose, hypercalcemic activity was detected. The compound had some drug-related toxicity at 5 μg/kg body weight dose. All higher doses tested were toxic and hypercalcemic in dogs. Although we observed drug-related toxicity in our preclinical toxicity studies, doses tested were significantly higher than those proposed for the phase I clinical trial.

2. Our results on competitive binding studies with VDR indicate that $1\alpha(OH)D_5$ has relatively lower binding affinity than 1,25(OH)2D5. These results suggest that $1\alpha(OH)D_5$ may possibly mediate its cell-differentiating and antiproliferative actions through VDR and also through other pathways.

3. We have established 4 different cell lines with different VDR and ER status. These cell lines are cloned and will be used to determine interaction between ER and VDR and the effect of $1\alpha(OH)D_5$ on these cells.

4. Studies on MDA-MB-231 (ER+, VDR+) cells clearly indicate that $1\alpha(OH)D_5$ influences ER expression in breast cancer cells.

5. We have further confirmed our previous findings that $1\alpha(OH)D_5$ inhibits proliferation and induces cell differentiation markers in breast tumors (tumors obtained from patients) in vitro.

6. We have prepared sufficient quantity of $1\alpha(OH)D_5$ under GMP for future clinical studies.

7. We are in the process of filing for FDA approval of the $1\alpha(OH)D_5$ phase I clinical trial for breast cancer.

Reportable outcomes

Publications:

1. Mehta R.R., Mehta R.G. Differentiation of human breast carcinoma cell line by a novel vitamin D analog: $1\alpha(OH)D_5$. Int J Oncology 16: 65-73, 2000.

2. Lazzaro G., Agadir A., Qing W., Poria M., Mehta R R. Moriarty R.M., Zhang X, Mehta R.G. Induction of differentiation by ı$1\alpha(OH)D_5$ in T47D human breast cancer cells and its interaction with vitamin D receptor. Eur J Cancer 36: 780-786, 2000.

3. Mehta R.G. and Mehta R.R. Vitamin D and cancer. Int J Nutr Biochem, 2001, In press.

Presentations at the national and international meetings:

1. Mehta R.R., Mehta R.G., Hussain E., Moriarty R., Mehta R.R. and Das Gupta T.K. Chemoprevention of mammary carcinogenesis by synthetic analog of vitamin D. Mutation Res. Seoul, Korea, 2002.

2. Johnson W.D., Mehta R.R., Moriarty R.M., Mehta R.G. Preclinical toxicity of $1\alpha(OH)D_5$ in rats and dogs. Proc Am Assoc Cancer Res 42:933, 2001.

3. Mehta R.R., Christov K and Mehta R.G. Effects of $1\alpha(OH)D_5$ are selective to malignant breast epithelial cells. Proc Am Asso Cancer Res 42:203, 2001.

4. Hussain E.A., Bhat K., Mehta R.R. and Mehta R.G. $1\alpha(OH)D_5$ induces apoptosis and cell cycle arrest in BT-474 breast cancer cells. Proc Am Assoc Cancer Res 42: 209, 2001.

Conclusions

We have completed preclinical toxicity studies in dogs under GMP. We have completed synthesis of $1\alpha(OH)D_5$ under GMP for future clinical trial. In vitro studies in clinical specimens obtained from women suggest that $1\alpha(OH)D_5$ has no effect on normal breast tissues; it inhibits cell proliferation in tumor cells. This implies that it has no bad effects on normal breast tissues but does inhibit cancer growth. $1\alpha(OH)D_5$ or its active metabolite possibly interacts with estrogen receptor. We will be submitting our IND application to the FDA.

Our findings to date imply that $1\alpha(OH)D_5$ has no bad effects on an overall biologic system (beagle dog) or on normal breast tissues but does inhibit cancer cell growth. It also appears that it might affect the estrogen cycle in cells (as do some already used anti-breast cancer agents). The fact that we are applying for approval to bring a vitamin derivative to clinical trial represents a very hopeful development in cancer treatment.

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Appendices

Appendix 1 Figures 1-4.

Appendix 2 Synthesis of $1\alpha(OH)D_5$ for clinical studies.

Appendix 3 A detailed preclinical toxicity report in dogs.

Appendix 4 Abstracts presented at 2001 annual AACR meeting.

Appendix 1: Figures 1-4.

Figure 1. Immunostaining for VDR in control plasmid only transfected MDA-MB-231 cells (a) and VDR cDNA transfected MDA-MB-231 cells (b).

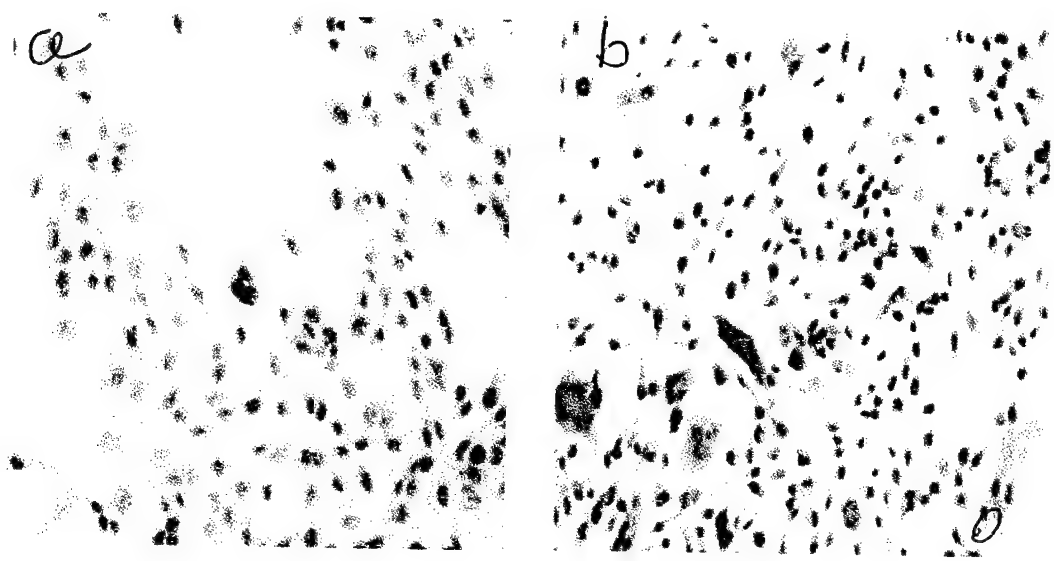

a
b

Figure 2. Immunostaining for VDR (Vitamin D receptor) in MDA-MB-231 cells transfected with plasmid DNA only (a,b,c) and MDA-MB-231 cells transfected with VDR cDNA. Cells were treated in vitro at 37c with vehicle containing culture medium (a d), 0.1μM 1,25 $(OH)_2$ D_3 (b,e) or 1μM 1α $(OH)D_5$ (c,e).

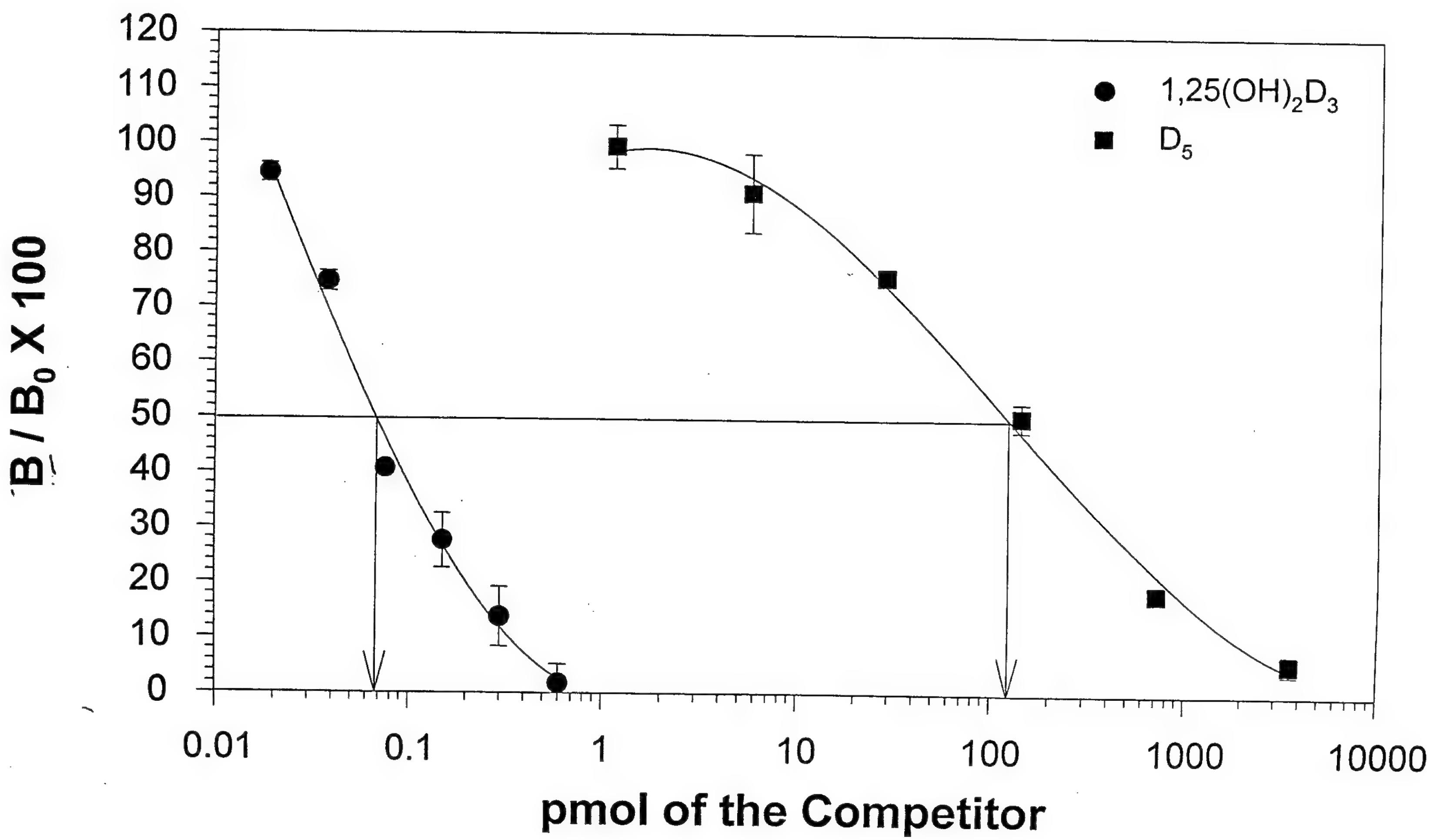

a
b
c
d
e
f

Figure 3. Immunostaining for ER in S-30 (ER cDNA transfected MDA-MB-231) transfected with plasmid DNA only (a, b, c) or cells transfected with VDR cDNA(e,f,g). Cells were treated for 48 hrs with vehicle containing medium (a,d), 1,25 $(OH)_2$ D_3 containing medium (b,e) and 1α $(OH)D_5$ containing medium (c,f).


a
b
c
d
e
f

Figure 4. Competition of 1α (OH)D_5 with 1,25 (OH)D_3 for vitamin D receptor (VDR). VDR ligand binding domain was incubated with radioactive 1,25 (OH)D_3 alone or with increasing molar concentration of non radioactive 1,25 $(OH)_2$ D_3 or 1α (OH)D_5.

Binding of Vitamin D_5 to VDR



Appendix 2: Synthesis of $1\alpha(OH)D_5$ for clinical studies.

Status Report of 1∝-OH vit-D_5

Following is the procedure at Conquest , Inc. to convert stigmasterol to 1a-hydroxyvitamin D5

Step 1 : Stigmasterol Tosylate :

Stigmasterol (50gms) was dissolved in pyridine (175 ml) and cooled in ice-bath to O-5°C. To this was added in several portions Tosyl chloride (43 gms) over a period of 0.5 hrs. The resulting solution was stirred at rt in dark for 20 hrs. Progress of the reaction was monitored by TLC (5 % Hex : EtoAc rf 0.5). The reaction mixt. was poured into cold 5% $NaHCO_3$ solution Wherein tosylate precipitated out. The solid was stirred for 15 min and filtered washed with water and air dried to yield stigmasterol tosylate in 64 gms.

Step 2: Preparation of stigmasterol methyl ether :

A suspension of stigmasterol tosylate (64 gms) potassium acetate (70 gms) and anhydrous methanol was refluxed for 5 hrs. The reaction was monitored by TLC (Rf = 0.7, 5% Hexane : EtOAc). MeOH was evoparated in vacuum, and ether was added and washed with water, 5 % NaHCO3, brine and dried over sodium sulphate. The solvent was concentrated in vacuo to afford 45 gms of methyl ether as a pale yellow viscous liquid.

Step 3: Preparation of sitosterol methyl ether :

A solution of stigmasterol methyl ether (10 gms) in ethyl acetate (250 ml) and 10 % Pd/C (3 gms) was stirred at rt under H_2 atmosphere using ballon for three days. The catalyst filtered through celite and the solvent was removed to afford the sitosterol methyl ether. The yield was 9.5 gms.

Step 4: Preparaion of sitosterol acetate :

A solution of sitosterol methyl ether (50 gms) in glacial acetic (1 ltr) acid was refluxed with Zinc acetate (65 gms) for 3 hrs. The reaction was monitored by TLC (Rf = 0.4, 5 % Hexane: EtOAc). Then the reaction mixture was cooled to rt , water was added. The resulting white ppt was filtered , washed with water and air dried. Recrystaillization from ether : methanol afforded sitosterol acetate. 42 gms as a colourless solid.

Step 5: Preparation of 7-Dehydrositosterol :

A suspension of sitosterol (1gms), anhydrous $NaHCO_3$ (0.9gms) and dibromontin in hexane (25 ml) was refluxed for 2 hrs. The reaction mixture was cooled to rt and filtered , and then the solvent was removed in vacuo. To the reaction flask, THF was added followed by tetrabutyl ammonium bromide (0.061 gms). The solution was stirred at rt for 30 minutes. To this reaction mixture was added tetrabutylammonium fluoride (2.92) and pyridine (0.5 ml). Then the reaction mixture was stirred at rt for 20 hrs. The crude reaction mixture was transferred to a separating funnel, water layer was removed, washed the organic layer with water, 1 N HCl, water and then brine. The organic layer was dried and concentrated in vacuo to afford a dark brown viscous liquid. The crude reaction mixture was purified by column chromatography (silica gel. Ethyl-hexane 1 :9 mixture as eluent) to afford 7-dehydrositosterol acetate as a semi-solid.

Step 6: Preparation of vitamin D5 acetate

7_Dehydrositosterolacetate (6.5 gms) and ethyl 4-dimethylamino benzoite (1.0gm) of diisopropylether:benzene were irradiated with a 450 W medium pressur mercury arc lamp at 5oC under nitrogen purging in a photochemical with quartz immersion well, after 4 hrs of irradiation, uranium filter was inserted and then 50 gm of 9-acetylanthracene was added and continued the irradiation for 1h and 15 min. The solution was then conc. Under pressure to afford the pre vit. D5 acetate (6.5 gm). The crude material was heated

in ethanol at 60oC for four hrs. with stirring in a water bath. The solvent was then evaporated under vacuo to afford vit D5 acetate as brown viscous compound (6.3gm).

Step 7: Preparation of vitamin D5

The crude vit. D5 acetate(6.3 gm) was dissolved in dry THF(250 mL) and cooled to 0oC under stirring. Lithium aluminum hydride(5.27 gm) was added slowly in several portions over 30 min. period and stirred at RT for 1.5 hrs. Progress of the reaction was monitored by TLC. The reaction was quenched by slow addition of water and diluted with ethyl acetate. The mixture was filtered through a celite and washed the residue with ethyl acetate and the combined solvents were evaporated to furnish crude vit. D5. Column purification of the same afforded 3.3 gm of the pure compound.

Step 8: Preparation of vitamin D5 tosylate

To a solution of vit. D5 (3.3 gm) in dry methylene chloride (100mL) was added triethyl amine (2.8mL) and cooled the mixture to 0oC. After 15 min of stirring tosylchloride (3.0gm) was added and brought the reaction mixture to room temperature and stirred for 3 hrs. the progress of the reaction was monitored by TLC. Then saturated sodium bicarbonate was added and extracted with dichloromethane and washed with brine and water and dried to afford the tosylate as syrapy compound (4.12g).

Step 9: Preparation of cycloviatmin D5

The above tosylate (5.0 gm) in methanol (180 mL) and saturated sodiumbicarbonate (41.1 gm) was refluxed for 5 hrs. Progress of the reaction was followed by TLC. Solvent was removed under vacuo and poured in to cold water and extracted the product in to dichloromethane. The organic layer was washed with brine solution and dried over sodium sulfate and evaporated to give the methyl ether (3.2 gm).

Step 10: Preparation of 1a-hydroxycyclovitamin D5

A mixture of selenium oxide (0.46 gm) and TBHP (1.48 gm) and dichloromethane (100 mL) was stirred for 3 hrs under nitrogen at room temperature. The mixture was cooled to 0oC and to it was added catalytic amount of pyridne. The methyl ether (3.2 gm) dissolved in dichloromethane (30 mL) was added dropwise over 15 min. period and stirred the mixture for 1 hr. The progess of the reaction was followed every 10 min. The crude mixture was purified by column chromatography to afford 1.1 gm of allyl alcohol derivative of vit. D5.

Step 11: Preparation of cis and trans mixture of 1a-hydrixyvitamin D5

The above compound (1.05gm) was stirred in a mixture of DMSO and acetic acid at 56 to 60oC under nitrogen on a water bath. After 1 hr. TLC showed the completion of the reaction. The mixture was poured into water and extracted with ethyl acetate and concentrated to afford 1.0 gm of the product.

Step 12: Preparation of 1a-hydroxyvitamin D5

A solution of the crude product (1.0 gm) and maleic anhydride (230 mg) and ethylacetate (160 mL) was stirred at room temperature for 24 hrs under nitrogen. The solvent was stripped off under vaccum and chromatographed over silica gel and eluted with ethyl acetate and hexanes to afford the product (500 mg). The product was further purified by reverse phase HPLC followed by crystallization from hexane to yield 350 mg of the 1a-hydroxyvitamin D5 with the purity greater than 98%.

Conclusion: The synthesis of 1α-hydroxy vitamin D5 involves several steps. The first seven steps of the synthesis were carried under non GMP and the last five steps were carried under GMP conditions. 350 mg of vitamin D5 was prepared under GMP conditions with the purity greater than 98%.

Appendix 3: A detailed preclinical toxicity report in dogs.

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

DRAFT REPORT

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IITRI Project No. 1209
Study No. 2

Testing Facility:

IIT Research Institute
Life Sciences Operation
10 West 35th Street
Chicago, IL 60616

Michael Reese Hospital
2929 South Ellis Avenue
Chicago, IL 60616

Authors:
William D. Johnson, Ph.D., D.A.B.T.
Study Director

David L. McCormick, Ph.D., D.A.B.T.
Principal Investigator

Sponsor:

University of Illinois at Chicago
Department of Surgical Oncology
840 South Wood Street
Chicago, IL 60612-7322

Sponsor Representative:
Tapas K. Das Gupta, M.D., Ph.D., D.Sc.

Study Completion Date: March 2001

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

SUMMARY

A 28-day oral toxicity study was conducted in male and female beagle dogs to evaluate the toxicity of 1α-Hydroxyvitamin D_5 when administered orally for four weeks and to determine the reversibility of any observed toxic effects. The test article, 1α-Hydroxyvitamin D_5 (1αD_5), was administered by oral gavage in a vehicle of corn oil initially at doses of 10, 30 and 90 µg/kg/day at a constant dosing volume of 1 ml/kg/day. A vehicle control group was administered an equivalent volume of vehicle (corn oil) only. The study design originally included 3 dogs per sex in the low and middle (10 and 30 µg/kg) dose groups and 5 dogs per sex in the vehicle control and high dose (90 µg/kg) groups, with 2 dogs/sex in the control and high dose groups being retained (untreated) for an additional two week period to determine recovery from any toxic effects. Because of toxicity (i.e., mortality of two female dogs and body weight loss of both male and female dogs) at the high dose (90 µg/kg) level during the first week of the study, the high dose recovery group was eliminated, and the high dose level for all surviving high dose dogs was decreased to 45 µg/kg for the remainder of the 28-day dosing period. In addition, the two dogs per sex in the vehicle control group originally designated as recovery animals were dosed with the test article at a level of 5 µg/kg for 28 days. Toxicological endpoints included physical examinations/clinical observations, ophthalmic examinations, body weights, food consumption, clinical pathology (hematology, clinical chemistry, urinalysis), organ weights and electrocardiographic evaluations. Tissues from all dogs in the vehicle control and 10 µg/kg dose groups, and from two dogs in the 30 µg/kg dose group which were sacrificed moribund were evaluated histopathologically. In addition, target tissues and gross lesions from dogs in the 5 µg/kg dose group were also evaluated histopathologically.

Administration of 1α-Hydroxyvitamin D_5 at dose levels greater than 5 µg/kg induced symptoms of hypervitaminosis. Eight dogs died or were sacrificed moribund during the study (2 females at 90 µg/kg; 3 males at 45 µg/kg, and 2 males and 1 female at 30 µg/kg). Drug-related clinical observations observed in animals at doses of 10 µg/kg and above consisted of thinness/emaciation, bloody salivation, hypothermia, dehydration, hypoactivity, labored breathing, lacrimation, conjunctivitis, ocular discharge and swollen cheeks.

Mean body weight and body weight gains were statistically significantly decreased by Day 8 such that, by the end of the 28-day treatment period, dogs treated at dose levels greater than 5 µg/kg had lost from 25 to 43% of their mean initial body weight. Body weight losses in these dogs were accompanied by decreased food consumption. Erythrocyte count, hematocrit and hemoglobin levels

were increased in both sexes at doses of 10 μg/kg and above, which most likely resulted due to the dehydrated condition of these animals. Serum calcium levels were increased (hypercalcemia) and serum inorganic phosphorus levels were significantly decreased in a dose-dependent manner in both sexes at all dose levels. In addition, females at dose levels of 30 μg/kg and higher had decreased alkaline phosphatase, along with increased blood urea nitrogen, cholesterol and triglyceride levels. Increased triglyceride levels were also seen in males at the 30 μg/kg dose level, while blood urea nitrogen levels were increased at the 10, 30 and 90/45 μg/kg dose levels in male dogs. Significantly decreased absolute organ weights (heart, liver, spleen, ovaries) and significantly increased relative organ weights (adrenals, brain, kidneys) were present in dogs at dose levels above 5 μg/kg, but were related to the severely decreased body weights of these animals, rather than indications of specific target organ toxicity. Significantly decreased absolute and relative thymus weights seen in these dogs were, however, drug-related. No treatment-related ophthalmic or electrocardiographic changes were seen in any dog at any dose level. Administration of 1α-Hydroxyvitamin$_{D5}$ at a dose of 10 or 5 μg/kg resulted in microscopic lesions in the kidney (tubule dilation, cortical mineralization, and basophilic tubules), mid-mucosal pyloric mineralization in the stomach, thymic atrophy (females only at 5 μg/kg), and hypertrophy/hyperplasia of thyroid parafollicular cells (females only at 5 μg/kg). Administration of 1α-Hydroxyvitamin $_{D5}$ at a dose of 10 μg/kg also resulted in mineralization in arteries of the spleen (females only) and heart (males only), bone marrow depletion, and cartilage hypoplasia in the femur. These lesions were all considered results of vitamin D metabolite activity or secondary to hypercalcemia induced by administration of the test article.

In conclusion, administration of 1α-Hydroxyvitamin$_{D5}$ at dose levels of 5, 10, 30 and 90/45 μg/kg via oral gavage daily for 28 days induced signs of hypervitaminosis D, which resulted in mortality at the 30 and 90/45 μg/kg dose levels. A no-observable-effect level (NOEL) was not established in this study, as serum calcium levels were increased at the 5 μg/kg dose level, and histopathological changes of minimal severity were seen in the kidneys, stomach, thymus and thyroid gland at the end of the 28-day dosing period in animals administered 1α$_{D5}$ at the 5 μg/kg dose level.

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Study Initiation Date:	September 5, 2000
Experimental Initiation Date:	September 5, 2000
Experimental Termination Date:	October 12, 2000

FOREWORD

This report describes a four-week oral (gavage) toxicity study in beagle dogs conducted by IIT Research Institute (IITRI) for the Department of Surgical Oncology, University of Illinois at Chicago. The Sponsor Representative for the study was Tapas K. Das Gupta, M.D., Ph.D., D.Sc.

William D. Johnson, Ph.D., D.A.B.T., served as Study Director and was responsible for the overall conduct of the study. David L. McCormick, Ph.D., D.A.B.T., Vice President and Director, Life Sciences Operation, served as Principal Investigator. J. Brooks Harder, D.V.M., IITRI staff veterinarian, was responsible for animal care. Jeff Kreyer, B.S. Associate Laboratory Biologist, served as Study Supervisor, responsible for animal dosing and data collection. Mary Ann Cahill, B.S., M.T. (A.S.C.P.), performed the clinical pathology evaluations. Michael J. Cwik, Ph.D., Senior Chemist, performed the analysis of the test article formulations. Robert L. Morrissey, Ph.D., D.V.M., D.A.C.V.P., of Pathology Associates International, Chicago, IL, served as the study pathologist. Ophthalmological evaluations were performed by Amy Hunkeler, D.V.M., Consultant (Animal Eye Associates). Electrocardiograms were evaluated by Michael W. Luethy, D.V.M., D.A.C.V.I.M., Consultant. John. G. Class, B.S., Manager, Quality Assurance, was responsible for the IITRI quality assurance program.

J. Fred Krueger, M.S., Senior Technical Editor, assisted in the preparation of this report.

William D. Johnson, Ph.D., D.A.B.T. Date
Study Director
Life Sciences Operation

David L. McCormick, Ph.D., D.A.B.T. Date
Vice President and Director
Life Sciences Operation

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

GLP COMPLIANCE STATEMENT

This study was conducted in accordance with the U.S. Food and Drug Administration (FDA) Good Laboratory Practice (GLP) Regulations as set forth in the *Code of Federal Regulations* (21 CFR Part 58) with the following exception: the vehicle for the study was corn oil; however, the bulk test article was first dissolved in a carrier of absolute ethanol. This stock solution was stored appropriately and dosing formulations were prepared therefrom. These stock $1\alpha D_5$/ethanol solutions were not analyzed for concentration, homogeneity or stability. The identity, purity and stability of the bulk test article were the responsibility of the Sponsor and a copy of the Certificate of Analysis provided is included in Appendix B of the report. The vehicle (corn oil) was a purchased product and, as such, was characterized by a Certificate of Analysis (Appendix B) provided by the vendor. The study raw data have been reviewed by the Study Director, who certifies that the information contained in this report accurately reflects and is supported by the data.

William D. Johnson, Ph.D., D.A.B.T. Date
Study Director
Life Sciences Operation

TABLE OF CONTENTS

TABLE OF CONTENTS (cont.)

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

I. INTRODUCTION

The objective of this study was to evaluate the toxicity of 1α-Hydroxyvitamin D_5 when administered orally to beagle dogs for four weeks, and, initially, to determine the reversibility of any observed toxic effects..

II. MATERIALS AND METHODS

A list of abbreviations used in this report and their definitions is given in Table 1. The study protocol, protocol amendments and protocol deviations are included as Appendix A.

A. Test Article and Vehicle: The test article, 1α-Hydroxyvitamin D_5 (1αD_5 ; lot 1AVD5-00A001), a white powder, was received in two shipments: the first on June 5, 2000 and the second (lot number not specified) on July 8, 2000. The test article was received in amber glass vials and was stored frozen (-60 to -80°C) in the original containers, protected from light and under a nitrogen atmosphere. Documentation of the identity, purity and stability of the bulk test article were the responsibility of the Sponsor. A Certificate of Analysis for this lot of test article, documenting identity and purity, is included in Appendix B. The vehicle (corn oil) used in this study was purchased from Sigma Chemical Co., St. Louis, MO (lot no. 89H0149) and was received on August 30, 2000 and stored at room temperature. A Certificate of Analysis for this lot is included in Appendix B. To facilitate dosing formulation preparation, bulk 1αD_5 was first dissolved in a carrier of absolute ethanol (McCormick Distilling Co., Weston, MO; Lot no. P287339; received July 2, 1998) to make stock solutions, aliquots of which were then used to prepare the dosing formulations. The Sponsor was responsible for archiving a retention sample of the bulk 1αD_5. A sample of the corn oil vehicle will be retained at IITRI. Remaining test article will be returned to the Sponsor at the completion of the study.

B. Test Article Formulation and Analysis: Doses (including the vehicle control) were intended to be administered to all dogs at a uniform dosing volume of 1 ml/kg of body weight. Dosing formulation concentrations were calculated to deliver intended doses (initially 10, 30 and 90 μg 1αD_5/kg of body weight and, later, 5, 10, 30 and 45 μg/kg) to the dogs. In order to facilitate dose formulation preparation, stock solutions were

prepared by dissolving bulk $1\alpha D_5$ in a carrier of absolute ethanol (ETOH). The first stock solution (34,875 μg/ml) was prepared 4 days prior to initiation of dosing. The original intent was to prepare dose formulations weekly using this stock solution; however, revision of the study design on Days 8-9 (see Section II.E.) required altering this proposed schedule. A second stock solution (18,750 μg/ml) was prepared 17 days later, subsequent to study design revision, and used for the duration of the study. Stock solutions were stored frozen (-60 to -80 °C) under nitrogen and protected from light and were used to prepare subsequent dosing and analytical formulations and standards. Dosing formulations initially at concentrations of 10, 30 and 90 μg/ml and, later in the study, at concentrations of 5, 10, 30 and 45 μg/ml in corn oil were prepared so as to deliver appropriate doses at dosing volume of 1 ml/kg. During the second week of the study, with the revision and addition of dose levels, the 10 μg/ml and 90 μg/ml dosing formulations were administered at 0.5 ml/kg dosing volumes to the 5 and 45 μg/kg dose groups, respectively. Starting with the third week of dosing (week 2 for the 5 μg/kg dose group), dosing at dose volumes of 1 ml/kg was resumed. All dosing formulations were stored refrigerated (approximately 4 °C) in amber jars prior to (blanketed under nitrogen) and during the week of dosing. The stability of the dosing formulations for one week under the conditions of use was verified. In addition, homogeneity of one dosing formulation (30 μg/ml) prepared for week 1 of dosing was determined and the concentrations of all dosing formulations used in this study were analyzed to verify the concentration of $1\alpha D_5$. Analytical methods used and results are detailed in Appendix B.

C. Animals, Housing and Diet: Beagle dogs used in this study were purchased from Ridglan Farms, Inc., Mt. Horeb, WI. The dogs were received August 23, 2000. The animals were between 5 and 6 months of age and weighed between 6.4 and 8.7 kg at the time of receipt. Their body weight range at the time of dosing initiation was 5.8 to 8.2 kg. The dogs were individually housed in stainless steel cages equipped with automatic watering and suspended over excrement pans. Dogs were housed in accordance with the *Guide for Care and Use of Laboratory Animals* (National Research Council, 1996) and the U.S. Department of Agriculture through the Animal Welfare Act (7 U.S.C. 2131-2156, 1985) and the Animal Welfare Standards incorporated in Title 9, CFR, Part 3, 1991. Each dog was identified by means of a USDA tattoo number in the right or left ear. A card containing the project number, study number, animal number, sex and group was also attached to each cage. All dogs were exercised daily during the quarantine and treatment periods to contribute to their physical and psychological well-

being. Animal room temperature and relative humidity values recorded daily during the quarantine and treatment periods were 19-28°C and 32-98%, respectively. The occasional brief excursions of temperature and relative humidity beyond the range limits specified in the protocol (18 to 26°C and 30 to 70% relative humidity) were not expected to significantly impact the outcome of the study. Fluorescent lighting in the animal room was provided for 12 hours followed by 12 hours of darkness.

Approximately 300 g of Purina Certified Canine Diet 5007 (PMI Feeds, Inc., St. Louis, MO) was offered daily for approximately two hours except on Day 1 (see Section II.F.5). Municipal water was available *ad libitum*. Based on analytical reports for the diet provided by the vendor and City of Chicago water analysis reports, no contaminants were known to be present in the food or water at levels expected to interfere with the outcome of the study.

D. Quarantine: Animals were held in quarantine for 13 (males) or 14 (females) days prior to dosing, during which time they were observed daily for survival and general health. A physical examination including clinical pathology, body weight and rectal temperature was performed on each dog once during the quarantine period . Animals were examined carefully to ensure their health and suitability as test subjects prior to assignment to experimental groups. Animals were randomly assigned to groups using a computerized randomization procedure that blocks for body weights.

E. Experimental Design: Dogs were initially assigned to four groups consisting of five, three, three and five dogs per sex per group. Initial dose levels were 0 (vehicle control), 10, 30 and 90 μg/kg/day. Three dogs per sex per group were scheduled to be sacrificed after 28 days of dosing, while the remaining 2 dogs/sex from the vehicle control and high dose groups were to be sacrificed following a two-week recovery period. The study design was as follows:

Group	1αD$_5$Dose (μg/kg body weight)	No. of Animals Main Study (M + F)	No. of Animals Recovery (M + F)
1	0 (Control)	3 + 3	2 + 2
2	10	3 + 3	--
3	30	3 + 3	--
4	90	3 + 3	2 + 2

Because of toxicity (i.e., mortality of two female dogs and body weight loss of both male and female dogs) at the high dose (90 μg/kg) level during the first week of the study, the high dose recovery group was eliminated, and the high dose level for all surviving high dose dogs was decreased to 45 μg/kg body weight for the remainder of the 28-day dosing period, beginning September 13, 2000 (study day 9 and 8 for males and females, respectively). In addition, the two dogs per sex in the vehicle control group originally designated as recovery animals were dosed with the test article at a level of 5 μg/kg for 28 days in order to obtain a no-observable-effect level (NOEL). Mortality of two high dose female dogs and dosing of the recovery control dogs with test article eliminated the recovery group animals. The modified study design was as follows:

Group	1αD_5 Dose (μg/kg body weight)	No. of Animals (M & F)
1	0 (Control)	3 + 3
2	10	3 + 3
3	30	3 + 3
4	45	5 + 3
5	5	2 + 2

To facilitate necropsy, dosing of male and female dogs was initiated over two days. Thus, treatment initiation (Day 1) was September 5, 2000 for males and September 6, 2000 for females. Dosing was scheduled for once per day for 28 days. However, mortalities in the high dose (90 μg/kg) dose group prompted suspension of dosing for one day in the high dose group (September 12, 2000; Day 8 for males and Day 7 for females). The dose level was decreased for this dose group from 90 to 45μg/k/day starting September 13, 2000 (Day 9 and 8 for males and females respectively) and dosing was continued at the lower level for a total of 28 doses. On the same day, the two recovery control males and females were switched to treatment with 5 μg 1αD_5/kg/day and treatment continued such that all test article-treated dogs received a total of 28 doses at designated dose levels, although not on 28 <u>consecutive</u> days as originally scheduled. Vehicle control dogs received a total of 37 (males) or 36 (females) doses. These design revisions resulted in the final days of treatment (Day 28) being October 2, 3, 4 and October 11, 2000. The following summarizes significant milestones of the study:

September 5, 2000 - initiation, Day 1, males;

September 6, 2000 - initiation, Day 1, females;

September 11, 2000 - Day 7 males, Day 6 females; decision to revise study design;

September 12, 2000 - Day 8 males, Day 7 females - high dose group not dosed;

September 13, 2000 - Day 9 males, Day 8 females - high dose lowered to 45 μg/kg and September 13, 2000 - Day 1 for Group 5 (5 μg/kg) males and females;

October 3, 2000 - Day 29 (terminal sacrifice) for surviving males in 10 and 30 μg/kg dose groups;

October 4, 2000 - Day 29 (terminal sacrifice) for surviving females in 10 and 30 μg/kg dose group and surviving males in 90/45 μg/kg dose group;

October 5, 2000 - Day 29 (terminal sacrificed) for surviving females in 90/45 μg/kg dose group;

October 11, 2000 - Day 29 (terminal sacrificed) for surviving males and females in the 5 μg/kg dose group and Day 37/36 (terminal sacrifice) for surviving vehicle control males/females.

F. Methods:

1. Test Article Formulation and Administration: The test article dosing formulations were prepared approximately weekly, except during the period of study design revision (week 2), as described above (Section II.B). Each formulation was prepared four days prior to use. The test article dosing formulations were prepared and stored at IITRI until use, when they were transported to the dog facility and stored refrigerated there during their week of use. Unused remnants were then returned to IITRI for disposition. Vehicle formulations were handled similarly. The vehicle and test article dosing formulations were removed from the refrigerator and warmed to room temperature prior to daily dosing. The dosing formulations were administered using a flexible polyethylene feeding tube and a plastic syringe. Animals received the test article or vehicle formulation by oral gavage at a constant dosing volume of 1 ml/kg of body weight (except during week 2 for the 90/45 μg/kg dose group and week 1 for the 5 μg/kg dose group), based upon each animal's most recently determined body weight. Gavage tubes were flushed with approximately 5 ml of tap water following dose administration.

2. Mortality/Moribundity Observations: Dogs were observed for moribundity and mortality twice daily during quarantine and during the dosing period (respective Days 1 - 28).

3. Physical Examinations, Clinical Observations and Body Temperatures: A physical examination was performed on each animal before assignment to a study group to ensure its suitability for use as a test animal. Complete physical examinations, including body temperature, were performed once during the quarantine period (pretest),prior to dose administration on study Day 1 (including the 5 µg/kg dose group with Day 1 = Day 13 for the other groups) and weekly thereafter during the dosing period. Animals were observed for adverse clinical signs daily during their respective 28-day dosing periods.

4. Body Weights: Body weights were measured once during the quarantine period (pretest), prior to dose administration (Day 1), and weekly thereafter during the respective 28-day dosing periods (Days 8, 15, 22 and 29).

5. Food Consumption: Food consumption was measured daily during the respective 28-day dosing periods. Dog chow (300 g) was offered for approximately 2 hours each day, except on Day 1 when the food was available to several dogs for less than 2 hours.

6. Ophthalmology: Indirect funduscopic examinations were performed on the eyes of all dogs during quarantine (pretest) and on all surviving dogs during the final week of the treatment period [Day 24; Day 17 (week 3 of treatment) for the 5 µg/kg dose group]. The cornea, iris, lens, fundus, and anterior and posterior chambers of the eye were evaluated and any lesions noted.

7. Electrocardiographic Evaluation: Electrocardiographic evaluations were performed on all dogs during the quarantine period (pretest) and on all surviving dogs during the last week of dosing.

8. Clinical Pathology: Blood samples for analysis of hematology, clinical chemistry and coagulation parameters were collected after an overnight fasting period during the quarantine period (pre-test) period and during the final week of treatment. Samples were collected from the jugular vein. Urine samples were also collected pre-test and at necropsy by catheterization for urinalysis. Hematological parameters evaluated using a Baker System 9000 analyzer (Biochem Immunosystems, Inc., Allentown, PA) consisted of erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count and total leukocyte count.

Fibrinogen, prothrombin time and activated partial thromboplastin time were measured using a MLA Electra 900 Automatic Coagulation timer (Hemoliance, Raritan, NJ). Hematological parameters evaluated microscopically consisted of red blood cell morphology, nucleated red blood cell count, differential white blood cell count (absolute and relative) and reticulocyte count (relative and absolute). The following chemistry parameters were evaluated using a Beckman Synchron CX5 analyzer (Beckman Instruments, Inc., Brea, CA): glucose, urea nitrogen, creatinine, total bilirubin, total protein, albumin (A), globulin (G), A/G ratio, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, cholesterol, triglycerides, lactate dehydrogenase, gamma glutamyl transpeptidase, sodium, potassium, calcium, inorganic phosphorus and chloride. Clinical pathology analyses were performed using LABCAT (IPA Inc., Princeton, NJ, version 4.43). Urinalysis parameters evaluated included volume, appearance, color, refractive index, specific gravity, pH, protein, glucose, bilirubin, urobilinogen, nitrite, ketones, leukocytes, occult blood and microscopic examination of sediment.

9. Necropsy: Complete necropsies were performed on all dogs, whether dying spontaneously, sacrificed moribund or sacrificed on the day of scheduled necropsy. On the day of moribund sacrifice or scheduled necropsy, dogs were sacrificed by an overdose of sodium pentobarbital and exsanguinated. The following tissues were collected and fixed in 10% neutral buffered formalin: adrenals, aorta (thoracic), brain, epididymides, esophagus, eyes (with optic nerves), femur (with head), gall bladder, heart, cecum, colon, duodenum, ileum, jejunum, rectum, kidneys, liver, lungs, lymph nodes (bronchial, mandibular and mesenteric), mammary gland, ovaries, pancreas, parathyroids, pituitary, prostate, mandibular salivary gland, sciatic nerve, skeletal muscle, skin (dorsal thorax, elbow), spinal cord (cervical and thoracic), spleen, sternum (bone marrow), stomach (fundic and pyloric regions), testes, thymus, thyroids, tongue, tonsils, trachea, ureter, urinary bladder, uterus, vagina and gross lesions. Adrenals, brain, heart, kidneys (separate), liver, ovaries, spleen, testes, thymus and thyroids (with parathyroids) were weighed for animals sacrificed at the terminal necropsy, and the organ-to-body-weight ratios were calculated. (Organs were also weighed for one female dog in the 30 μg/kg dose group sacrificed moribund one day prior to scheduled terminal necropsy.)

10. Histopathology: All fixed tissues from all dogs in the vehicle control (Group 1; 0 µg/kg) and low-mid (Group 2; 10 µg/kg) dose groups, and from two dogs (animal numbers 1261 male and 1239 female) in the high-mid (Group 3; 30 µg/kg) dose group which were sacrificed moribund were processed by routine histopathological methods, stained with hematoxylin and eosin and evaluated microscopically by a board-certified veterinary pathologist. In addition, target tissues and gross lesions from dogs in the low dose group (Group 5; 5 µg/kg) were also evaluated histopathologically.

G. Statistical Procedures: Body weight, body weigh gain, daily food consumption, clinical pathology (except urinalysis) and organ weight data were compared by analysis of variance followed, where appropriate, by the *post hoc* Dunnett's test. Emphasis was placed on comparing data after an equivalent number of doses, rather than on "time on test". Consequently, data from the 10, 30 and 90/45 µg/kg dose groups were compared with those from the vehicle control group at similar dosing intervals. Data from the 5 µg/kg dose group were compared with a separate set of vehicle control data collected at similar dosing intervals. The exception to this was organ weight data, wherein the comparison was all groups versus the vehicle control group as a whole. Comparisons were performed using Systat (SPSS, Inc, Chicago, IL, version 5.0) software, with a $p \leq 0.05$ considered significant in all cases.

H. Archives: All raw data generated at IITRI, specimens and a copy of the final report will be retained in the IITRI archives for a period of five years from the date of completion of the study. At that time, the Sponsor will be consulted concerning the final disposition of the archival materials.

III. RESULTS

A. Test Article Formulation Analysis: Results of the concentration, homogeneity and stability analyses of the test article formulations are presented in Appendix B. Analysis of the 30 µg/ml dosing formulation showed it to be homogenous (R.S.D. = 2%), while the analyzed concentration of all dosing formulations was within 20% of the target concentration. Stability analysis showed the dosing formulations to be stable for the duration of the one-week dosing period (99-109% of initial concentration).

B. Mortality, Clinical Observations and Physical Examinations: Mortalities and clinical observations are summarized in Tables 2 and 3, respectively. Individual animal physical examination data are presented in Table 4, while individual clinical observations are presented in Appendix C Table C-1. A total of eight mortalities occurred during the study, two males and one female in the 30 µg/kg dose group (two moribund sacrifice and one found dead) and three males and two females in the 90/45 µg/kg dose group (one moribund sacrifice and four found dead). Two females in the high dose group died after 5 and 6 doses, respectively, at the 90 µg/kg dose level. The others died after 23 or 26 doses (8 doses at 90 µg/kg and 15 or 18 doses at 45 µg/kg). The three dogs in the 30 µg/kg dose group died after 23 or 27 doses. All of the deaths were considered drug-related.

Drug-related clinical signs and physical examination findings in dogs dosed with 1α-Hydroxyvitamin D_5 at dose levels greater than 5 µg/kg included thinness/emaciation, emesis, bloody salivation , coldness to the touch, dehydration, hypoactivity, labored breathing, lacrimation, conjunctivitis, ocular discharge and swollen cheeks. Body temperatures of these animals dropped to below 100°F as the impact of dosing became more apparent. Most of the observations were observed in the groups dosed at the 30 and 90/45 µg/kg dose levels. Diarrhea was seen in all groups, including the vehicle control, although the incidence was higher in the 90/45 µg/kg males. Animals dosed at the 5 µg/kg dose level did not exhibit any treatment-related clinical signs of toxicity.

C. Body Weights: Mean body weights and body weight gains are summarized in Tables 5 and 6, respectively, and individual animal body weights and body weight gains are presented in Appendix C Tables C-2 and C-3. Mean body weights are also graphically depicted in Figures 1 and 2. Mean body weights of all drug-treated dogs (both sexes) at dose levels greater than 5 µg/kg decreased continuously for the duration of the study and were significantly decreased compared to vehicle controls beginning on Day 8 [10 (males only), 30 and 90 µg/kg dose groups] or 15 (10 µg/kg females) and for the duration of the study. Mean body weight gains were significantly decreased from the vehicle control group in both sexes at the 10, 30 and 90/45 µg/kg dose levels on Day s 8, 15, 22 and 29. Overall mean body weight losses were 27% and 25%, 34% and 43%, and 35% and 39% for males and females in the 10, 30 and 90/45 µg/kg dose groups, respectively. Animals of both sexes in

the 5 μg/kg dose group gained weight overall, although they did not gain as much weight as their vehicle control counterparts (0.36 and 0.34 kg versus 0.89 and 0.70 kg for males and females, respectively).

D. Food Consumption: Mean daily food consumption data are summarized in Table 7 and individual animal daily food consumption data are presented in Appendix C Table C-4. Mean daily food consumption declined in a dose-related fashion in drug-treated dogs (both sexes) shortly after dosing initiation. The decreases, compared to vehicle controls, were consistently statistically significant beginning on Day 3 in the 90/45 μg/kg (both sexes) and 30 μg/kg (females only) dose group, on Day 5 in the 30 μg/kg males and Day 9 and 8 for the 10 μg/kg males and females, respectively. By the end of treatment, all dogs treated at these dose levels exhibited severe appetite loss (< 5 g of food consumed on one or more days) and generally ate less than 100 g/day during the latter two weeks of dosing. In contrast, dogs in the 5 μg/kg dose group often ate more than their vehicle control counterparts, although there were no statistically significant differences except an increase in male dogs in the 5 μg/kg dose group on Day 2.

E. Ophthalmology: An ophthalmology report is included as Appendix D. No drug-induced ocular lesions were seen in any dog.

F. Hematology: Mean pre- and post-dose hematology and coagulation data are summarized in Tables 8 through 11. Individual animal hematology and coagulation data and red blood cell morphology observations are presented in Appendix C Tables C-5 through C-10. After four weeks of dosing, statistically significant changes in hematology parameters in dogs treated with $1\alpha D_5$ at dose levels greater than 5 μg/kg consisted of increased erythrocyte count (90/45 μg/kg males and females and 30 μg/kg females), increased hemoglobin and hematocrit (all dose levels, although hematocrit not statistically significant in 10 μg/kg males), and significantly decreased reticulocytes (absolute and relative) in 90/45 μg/kg males. Mean absolute and relative eosinophil counts were significantly increased in 10 μg/kg females, but, in the absence of a dose-related trend, the change was not considered treatment-related. Mean activated partial thromboplastin time (APTT) was significantly increased in females in the 30 and 90/45 μg/kg dose groups and fibrinogen levels were increased in the 90/45 μg/kg females. Mean APTT levels were also increased in the 90/45 and 30 μg/kg male dogs; however, the increases

were not statistically significant, most likely related to the small number of dogs in the group (30 μg/kg) or the large standard deviation (90/45 μg/kg dose group) resulting from the failure of the blood from one dog in this group to clot (animal number 1253; APTT value of 106 seconds). The only hematological change observed in 5 μg/kg dose group animals post-dose was a significantly increased fibrinogen level in female dogs compared to the vehicle control. This increase was not, however, considered treatment-related because of the lack of an effect in the female dogs at the 10 and 30 μg/kg dose levels. There were no readily apparent changes in red blood cell morphology observations in any dogs treated with $1\alpha D_5$ compared to the vehicle controls at the end of treatment.

G. Clinical Chemistry: Mean pre- and post-dose clinical chemistry data are summarized in Tables 12 and13. Individual animal clinical chemistry data are presented in Appendix C Tables C-11 and C-12. Statistically significant changes in male dogs treated with $1\alpha D_5$ at dose levels greater than 5 μg/kg consisted of increased calcium (hypercalcemia) and decreased inorganic phosphorus (all dose levels) and increased triglycerides (30 μg/kg dose level only). Blood urea nitrogen levels were also increased in a dose-dependent manner in male dogs at the 10, 30 and 90/45 μg/kg dose levels, although the increases were not statistically significant compared to the vehicle control group. Female dogs at all dose levels greater than 5 μg/kg also exhibited significantly increased calcium and decreased inorganic phosphorus values, as well as decreased alkaline phosphatase, increased blood urea nitrogen (not statistically significant in the 10 μg/kg dose group), and increased triglycerides (statistically significant only at the 90/45 μg/kg dose level). Calcium levels were increased up to 53% in the 30 μg/kg males and up to 58% in the high dose females, while inorganic phosphate levels were decreased 25% and 28% in the high dose males and females, respectively. Mean lactate dehydrogenase activity level was also significantly increased in females in the 30 μg/kg dose group, but, in the absence of a clear dose-related trend, the change was not considered treatment-related. Female dogs also appeared to have a dose-related increase in cholesterol, but the differences from the vehicle control group were not statistically significant.

For the dogs treated at the 5 μg/kg dose level, the only statistically significant changes observed compared to vehicle controls were decreased chloride and

inorganic phosphorus (15%) levels in females. The decreased chloride level was not considered treatment-related due to the lack of a dose response; however, the decreased inorganic phosphorus level observed in the 5 μg/kg dose group females was considered dose-related. Calcium levels were also increased in both males (15%) and females (14%), while inorganic phosphorus levels were also decreased (16%) in males at the 5 μg/kg dose level. Although these changes were not significantly different from the vehicle control group values, the changes were considered treatment-related.

H. Urinalysis: Individual animal urinalysis data are presented in Appendix C Tables C-14 and C-15. A key is included in the appendix (Appendix C Table C-13) to facilitate interpretation of the data. No treatment-related effects on urinalysis parameters were observed.

I. Electrocardiographic Evaluations: A summary of the electrocardiographic evaluations performed pretest and during the last week of dosing on each animal is included as Appendix E. No evidence of cardiovascular toxicity was observed in male or female dogs at the end of the 4-week dosing period.

J. Organ Weights: Mean absolute and relative (organ-to-body weight ratios) organ weight data are presented in Tables 14 and 15, respectively, and individual animal data are presented in Appendix C Tables C-16 and C-17. Treatment-related, statistically significant decreases in absolute organ weights were observed in animals (both sexes) administered $1\alpha D_5$ at all dose levels greater than 5 μg/kg and consisted of decreases in heart, liver and thymus weights. In addition, female dogs exhibited decreased absolute ovary weight at all three dose levels (10, 30 and 90/45μg/kg) and absolute spleen weight was decreased in male and female dogs at the 90/45 μg/kg dose level and in females at the 30 μg/kg dose level. The only statistically significant change with regard to absolute organ weight observed at the 5 μg/kg dose level was decreased ovary weight. The severely decreased body weights of animals dosed at levels greater then 5 μg/kg impacted the relative organ weights (organ-to-body weight ratios) of these animals, resulting in statistically significant increases in relative adrenal (all three dose levels; both sexes), brain (all three dose levels, both sexes), kidney (all three dose levels, females only; kidney weight in males was also increased, but the increases were not statistically significant), spleen (10 μg/kg females only) and thyroid (90/45 μg/kg females

only). The fact that the relative weights of organs with significantly decreased absolute weights (heart, liver, spleen and ovaries) were not significantly different from vehicle controls indicated that the significantly diminished size of those organs was a function of the overall loss of body weight observed during the study, and were not a result of overt target organ toxicity. However, relative thymus weight remained significantly decreased in 10, 30 and 90/45 μg/kg treated males and females, even after correction for diminished body weight, thus indicating a direct treatment-related effect on that organ. There were no statistically significant changes with regard to relative organ weights in male or female dogs at the 5 μg/kg dose level.

K. Gross Necropsy Observations: Gross necropsy findings are presented in Table IV of Appendix G (Pathology Report). Pigmentation changes were observed in the lung, kidney, stomach, spleen and intestines of animals dosed at 10, 30 and 90/45 μg/kg at higher incidences than in the vehicle control and 5 μg/kg dose groups. Small thymus was observed in all dogs at the 10, 30 and 90/45 μg/kg dose levels. Pigmentation changes were the result of the general debilitated condition of the animals, while small thymus correlated with a microscopic diagnosis of atrophy.

L. Histopathology: A detailed pathology report is included as Appendix G. Treatment- related microscopic lesions are summarized in Table III of the pathology report. Tissues from all vehicle control (3 males/3 females), 5 (2 males/2 females; target tissues only) and 10 (3 males/3 females) μg/kg dose group animals and two dogs (one male, one female) in the 30 μg/kg dose group which were sacrificed moribund were evaluated microscopically. Tissues from dogs that were found dead and those sacrificed moribund in the high dose (90/45 μg/kg) group were not considered suitable for processing and evaluation. Drug-related microscopic lesions were observed in the kidneys (tubule dilatation, cortical mineralization and diffuse basophilic tubules), stomach (mid-mucosal mineralization of the pyloric region), bone (hypoplasia of femoral epiphyseal cartilage), bone marrow (sternal and femoral, depletion), thymus (atrophy), heart (mineralization at the base of the aorta), skeletal muscle (atrophy, degeneration and subacute inflammation), spleen (mineralization of splenic artery), thyroid (hypertrophy/hyperplasia of parafollicular cells), parathyroid (hypertrophy), uterus

(atrophy), adrenal gland (focal mineralization and vacuolation of the cortex) and skin (abscess and ulceration). Most of these lesions were observed only in the 10 and 30 µg/kg dose group animals (both sexes), with dose-related increases in severity. Microscopic lesions that were also observed at the 5µg/kg dose level [kidney- tubule dilatation, cortical mineralization, basophilic tubules; stomach - mid-mucosal pyloric mineralization; thymus - atrophy (females only); thyroid - hypertrophy/hyperplasia of parafollicular cells (females only)], although of lesser severity and/or incidence were, nonetheless, interpreted as drug-related findings. Many of these lesions were associated or secondary to the hypercalcemia induced by and other vitamin D metabolite activity of the test article.

IV. DISCUSSION AND CONCLUSION

Administration of 1α-Hydroxyvitamin D_5 once daily for 28 days via oral gavage at dose levels of 10, 30 and 90 µg/kg resulted in mortalities at the 30 and 90 µg/kg dose levels. The early deaths at the 90 µg/kg dose level prompted the reduction of that dose level to 45 µg/kg after 8 (males) or 7 (females) days of treatment. The drug was observed to induce hypervitaminosis at these levels. Clinical observations in these dogs consisted of thinness/emaciation, bloody salivation, hypothermia, dehydration, hypoactivity, labored breathing, lacrimation, conjunctivitis, ocular discharge and swollen cheeks. Mean body weight gains were significantly decreased from the vehicle control group (animals actually lost weight, some with body weight loss of up to approximately 50% of predose weight) in both sexes at dose levels of 10 µg/kg and greater. Body weight losses in these dogs were accompanied by decreases in daily food consumption. Increases in erythrocyte count, hemoglobin and hematocrit were seen in both sexes at the 10, 30 and 90/45 µg/kg dose levels at the end of the 28-day dosing period. These increases most likely were a result of hemoconcentration of the blood due to the dehydration in these animals, rather than an indication of direct drug toxicity. Increased serum calcium (hypercalcemia) and decreased serum inorganic phosphorus levels were seen at all dose levels (including 5 µg/kg) in a dose-dependent manner. Changes in serum alkaline phosphatase (decreased), blood urea nitrogen (increased), cholesterol (increased) and triglycerides (increased) were also seen in female dogs at dose levels of 10 µg/kg and higher. Blood urea nitrogen levels were also increased in male dogs at dose levels of 10 µg/kg and above, while increased triglyceride levels were also seen in males at the 30 µg/kg dose level. The absolute weight of the heart, liver (both sexes), spleen and ovaries was significantly decreased at dose levels above 5 µg/kg;

however, organ-to-body weight ratios for these organs were not decreased, indicating these decreases were a function of the overall decreased body weight of the animals. Similarly, relative organ weights of other organs were increased (adrenals, brain, kidneys) solely as a function of diminished body weight at the 10, 30 and 90/45 μg/kg dose levels. Thus, these organ weight effects were not considered indicative of specific target organ toxicity. With regard to the thymus, however, absolute and relative thymus weights were significantly decreased in males and females at the 10, 30 and 90/45 μg/kg dose levels. Although considered drug-related, decreased thymus weight in these animals was probably related to generalized stress, rather than an indication of target organ toxicity. Drug-related microscopic lesions were observed in the kidneys (tubule dilatation, cortical mineralization and diffuse basophilic tubules), stomach (mid-mucosal mineralization of the pyloric region), bone (hypoplasia of femoral epiphyseal cartilage), bone marrow (sternal and femoral, depletion), thymus (atrophy), heart (mineralization at the base of the aorta), skeletal muscle (atrophy, degeneration and subacute inflammation), spleen (mineralization of splenic artery), thyroid (hypertrophy/hyperplasia of parafollicular cells), parathyroid (hypertrophy), uterus (atrophy), adrenal gland (focal mineralization and vacuolation of the cortex) and skin (abscess and ulceration). All of these lesions were associated with or secondary to the hypercalcemia induced by and other vitamin D metabolite activity of the test article, and appeared to exhibit a dose-response with regard to severity.

Evidence of mortality and/or toxicity in animals at the 10, 30 and 90 μg/kg dose levels resulted in the lowering of the high dose level from 90 to 45 μg/kg and the transfer of two dogs/sex from the vehicle control to a drug treatment group dosed with 1α-Hydroxyvitamin D_5 at 5 μg/kg, beginning after approximately one week of treatment and continuing for 28 days. None of the drug-related effects with regard to clinical observations, body weight and gain, daily food consumption, hematological parameters, and organ weights observed in dogs dosed at the higher levels were observed in the animals dosed at the 5 μg/kg dose level. However, serum calcium levels were increased and inorganic phosphate levels were decreased in dogs at the 5 μg/kg dose level, although the only statistically significant change was phosphate levels in the females. In addition, microscopic evidence indicated effects of drug treatment in the 5 μg/kg animals consisting of lesions in the kidney (tubule dilatation, cortical mineralization and basophilic tubules), stomach (mid-mucosal mineralization of the pyloric region), thymus (atrophy in the female dogs only) and thyroid (hypertrophy/hyperplasia of parafollicular cells in the female dogs only). These lesions,

however, were of lesser severity (generally minimal) in these animals than in those dosed at the 10 and 30 µg/kg dose levels.

In conclusion, administration of 1α-Hydroxyvitamin D_5 at dose levels of 5, 10, 30 and 90/45 µg/kg via oral gavage daily for 28 days induced signs of hypervitaminosis D, which resulted in mortality at the 30 and 90/45 µg/kg dose levels. A no-observable-effect level (NOEL) was not established in this study as serum levels of calcium were increased at the 5 µg/kg dose level, and histopathological changes of minimal severity were seen in the kidneys, stomach, thymus and thyroid gland at the end of the 28-day dosing period in animals administered 1α-Hydroxyvitamin D_5 at the 5 µg/kg dose level.

III. QUALITY ASSURANCE STATEMENT

Study Title:	Four-Week Oral (Gavage) Toxicity Study of 1α-Hydroxyvitamin D_5 in Beagle Dogs
Project Number:	1209
Study Number:	2
Study Director:	William D. Johnson, Ph.D., D.A.B.T.

The portions of this study conducted by IITRI have been subjected to inspections and the report has been audited by the IITRI Quality Assurance Unit in accordance with the U.S. Food and Drug Administration (FDA) "Good Laboratory Practice (GLP) Regulations" - "CFR Title 21 Section 58.35". The report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study. All raw data, specimens and a copy of the final report will be stored in the IITRI archives (10 West 35th Street, Chicago, IL) for a period of five years from the date of completion of the study.

The following are the inspection dates, and the dates inspection findings were reported:

	Inspection Findings Reported to:	
Dates of Inspections	Study Director	Management

John G. Class, B.S. Date
Manager, Quality Assurance Unit

VI. TABLES

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 1

Abbreviations

ALB	- albumin (grams / deciliter serum)
A/G RATIO	- albumin / globulin ratio
ALP	- alkaline phosphatase (international units / liter serum)
ALT	- alanine aminotransferase (international units / liter serum)
APTT	- activated partial thromboplastin time (seconds)
AST	- aspartate aminotransferase (international units / liter serum)
BAND NEU	- band cell neutrophils (absolute: thousands of cells / cubic millimeter blood; relative: percent leukocytes counted)
BASO	- basophils (absolute: thousands of cells / cubic millimeter blood; relative: percent leukocytes counted)
BUN	- blood urea nitrogen (milligrams nitrogen / deciliter serum)
CA	- calcium (milligrams / deciliter serum)
CHOL	- cholesterol (milligrams / deciliter serum)
CK	- creatine kinase (international units / liter serum)
CL	- chloride (millimoles / liter serum)
CREA	- creatinine (milligrams / deciliter serum)
dL	- deciliter
EOSIN	- eosinophils (absolute: thousands of cells / cubic millimeter blood; relative: percent leukocytes counted)
F	- female
g	- grams
GGT	- gamma glutamyl transpeptidase (international units / liter serum)
GLOB	- globulin (grams / deciliter serum)
GLU	- glucose (milligrams / deciliter serum)
HCT	- hematocrit (percent)
HGB	- hemoglobin (grams / deciliter blood)
IU	- international units
K	- potassium (millimoles / liter serum)
kg	- kilograms
L	- liter
LDH	- lactate dehydrogenase (international units / liter serum)
LYMPH	- lymphocytes (absolute: thousands of cells / cubic millimeter blood; relative: percent leukocytes counted)
M	- male
MCH	- mean corpuscular hemoglobin (picograms)
MCHC	- mean corpuscular hemoglobin concentration (percent)
MCV	- mean corpuscular volume (fl=femtoliter; 10^{-15} liter, equivalent to a cubic micron)
mg	- milligrams
mmol	- millimoles
MONO	- monocytes (absolute: thousands of cells / cubic millimeter blood; relative: percent leukocytes counted)
NA	- sodium (millimoles / liter serum)
NRBC	- nucleated red blood cells (number / 100 white blood cells)
PO4	- phosphorus (inorganic; milligrams / deciliter serum)
PLT	- platelet count (thousands / cubic millimeter blood)
PT	- prothrombin time (seconds)
RBC	- red blood cell count (millions of cells / cubic millimeter blood)
RETABS	- absolute reticulocyte count (thousands / cubic millimeter blood)
RETPC	- relative reticulocyte count (percent of total erythrocyte count)
SD	- standard deviation

Table 1 (cont.)

Abbreviations

SEG NEU	- segmented neutrophils (absolute: thousands of cells / cubic millimeter blood; relative: percent leukocytes counted)
TBIL	- total bilirubin (milligrams / deciliter serum)
TP	- total protein (grams protein / deciliter serum)
TG	- triglycerides (milligrams / deciliter serum)
VCTL	- vehicle control
WBC	- white blood cell count (thousands of cells / cubic millimeter blood); corrected for nucleated red blood cells

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 2

Summary of Mortality Data

Dose Group (μg/kg)	Number of Mortalities	Animal Number	Sex	Death	Day
1(VCTL; 0)	None	--	--	--	--
5 (5)	None	--	--	--	--
2 (10)	None	--	--	--	--
3 (30)	2 males and 1 female	1259	M	Found dead	Day 24
		1261	M	Moribund sacrifice	Day 24
		1239	F	Moribund sacrifice	Day 28
4 (90/45[a])	3 males and 2 females	1251	M	Found dead	Day 27
		1253	M	Moribund sacrifice	Day 23
		1255	M	Found dead	Day 23
		1247	F	Found dead	Day 7
		1248	F	Found dead	Day 6

[a] dose decreased from 90 to 45 μg/kg on Day 9 (males) or Day 8 (females)

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 3

Summary of Frequency[a] of Daily Clinical Observations - Males

Observation	Group: Dose (μg/kg):	1 VCTL; 0	2 10	3 30	4 90/45[b]	5 5
Terminal Sacrifice		3	3	1	2	2
Moribund Sacrifice		--[c]	--	1	1	--
Found Dead		--	--	1	2	--
Bloody Salivation		--	1	3	1	--
Cold To Touch		--	--	1	4	--
Dehydrated		--	--	--	1	--
Diarrhea		1	2	2	5	2
Emaciated		--	--	3	5	--
Emesis (Bile)		--	1	--		--
Hypoactive		--	--	3	5	--
Labored Breathing		--	--	--	1	--
Lacrimation		--	--	--	1	--
Ocular Discharge		--	--	--	1	--
Swollen Cheeks		--	1	2	1	--
Thin		--	1	--	1	--
	Total Number of Animals:	3[d]	3	3	5	2

[a] frequency = number of animals exhibiting the sign at some time during the study
[b] dose decreased from 90 to 45 μg/kg on Day 9
[c] -- = zero incidence
[d] 5 animals until Day 8; two animals moved to Group 5 on Day 9

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 3 (cont.)

Summary of Frequency[a] of Daily Clinical Observations - Females

Observation	Group: / Dose (μg/kg):	1 / VCTL; 0	2 / 10	3 / 30	4 / 90/45[b]	5 / 5
Terminal Sacrifice		3	3	2	3	2
Moribund Sacrifice		--[c]	--	1	--	--
Found Dead		--	--	--	2	--
Cold To Touch		--	--	3	--	--
Diarrhea		2	1	1	2	--
Emaciated		--	--	3	3	--
Emesis (Bile)		1	--	1	3	--
Hypoactive		--	--	3	--	--
Thin		--	2	--	3	--
	Total Number of Animals:	3[d]	3	3	5	2

[a] frequency = number of animals exhibiting the sign at some time during the study
[b] dose decreased from 90 to 45 μg/kg on Day 9
[c] -- = zero incidence
[c] 5 animals until Day 8; two animals moved to Group 5 on Day 8

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 4

Individual Animal Weekly Physical Examination Data - Males
Group 1 - Vehicle Control - 0 μg/kg

Day	Animal Number				
	1252	1256	1258	1263	1266
-4	102.8[a]; NVA[b]	102.3; NVA	101.2; NVA	102.1; NVA	101.7; NVA
1	101.6; NVA	101.8; NVA	101.2; NVA	101.7; NVA	101.7; NVA
8	100.4; NVA	101.9; NVA	101.1; NVA	101.8; NVA	101.4; NVA
15	100.7; NVA	101.5; NVA	Moved[c]	101.4; NVA	Moved
22	100.4; NVA	100.6; NVA	Moved	101; NVA	Moved
29	102.0; NVA	102.5; NVA	Moved	102.1; NVA	Moved

	1252	1256		1263
1	100.4; NVA	101.9; NVA		101.8; NVA
8	100.7; NVA	101.5; NVA		101.4; NVA
15	100.4; NVA	100.6; NVA		101; NVA
22	102.0; NVA	102.5; NVA		102.1; NVA
29	101.3; NVA	101.8; NVA		100.9; NVA

[a] Body temperature, °F
[b] NVA = no visible abnormalities
[c] Moved = began dosing with 5 μg 1α-Hydroxyvitamin D_5/kg on Day 9

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 4 (cont.)

Individual Animal Weekly Physical Examination Data - Males
Group 2 - Low - 10 µg/kg

Day	Animal Number		
	1257	1260	1262
-4	103.3[a]; NVA[b]	101.7; NVA	102.1; NVA
1	102.0; NVA	101.4; NVA	101.4; NVA
8	101.2; NVA	101.9; NVA	101.7; NVA
15	102.1; NVA	102.2; NVA	99.6; NVA
22	98.9; NVA	98.9; NVA	99.3; NVA
29	102.0; NVA	100.7; NVA	101.4; Emaciated; Cheeks swollen; Bloody saliva

[a] Body temperature, °F
[b] NVA = no visible abnormalities

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 4 (cont.)

Individual Animal Weekly Physical Examination Data - Males
Group 3 - Mid - 30 µg/kg

Day	Animal Number		
	1259	1261	1265
-4	100.7[a]; NVA[b]	101.5; NVA	102.8; NVA
1	100.9; NVA	101.3; NVA	101.2; NVA
8	100.5; NVA	100.8; NVA	101.3; NVA
15	98.1; NVA	99.0; NVA	101.6; NVA
22	100.1; Emaciated; Cheeks swollen; Bloody saliva	98.5; NVA	101.8; NVA
29	Dead	Dead	101.5; Thin

[a] Body temperature, °F
[b] NVA = no visible abnormalities

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 4 (cont.)

Individual Animal Weekly Physical Examination Data - Males
Group 4 - High - 90/45[a] μg/kg

Day	Animal Number				
	1251	1253	1254	1255	1266
-4	102.8;[b] NVA[c]	101.6; NVA	102.6; NVA	102.0; NVA	102.5; NVA
1	102.1; NVA	101.0; NVA	101.9; NVA	100.3; NVA	102.0; NVA
8	101.2; NVA	100.8; NVA	101.0; NVA	99.8; Listless; Rough hair coat	101.7; Bilateral ocular discharge
15	100.7; NVA	98.6; Emaciated	100.0; NVA	98.4; Emaciated	100.8; NVA
22	97.2; Emaciated	95.1; Emaciated	99.6; Emaciated	93.5; Emaciated	99.5; Emaciated
29	Dead	Dead	98.5; Emaciated	Dead	98.9; Emaciated; Ocular discharge; Cheeks swollen; Bloody saliva

[a] dose decreased from 90 to 45 μg/kg on Day 9
[b] Body temperature, °F
[c] NVA = no visible abnormalities

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 4 (cont.)

Individual Animal Weekly Physical Examination Data - Males
Group 5 - Low-Low - 5 µg/kg

Day	Animal Number	
	1258	1266
1	101.1[a]; NVA[b]	101.3; NVA
8	101.8; NVA	101.9; NVA
15	100.6; NVA	101.2; NVA
22	101.0; NVA	101.6; NVA
29	100.8; NVA	101.0; NVA

[a] Body temperature, °F
[b] NVA = no visible abnormalities

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 4 (cont.)

Individual Animal Weekly Physical Examination Data - Females
Group 1 - Vehicle Control - 0 μg/kg

Day	Animal Number				
	1235	1236	1244	1245	1249
-5	101.8[a]; NVA[b]	101.7; NVA	102.8; Conjunctivitis (right eye)	101.2; NVA	100.6; NVA
1	100.7; NVA	100.9; NVA	100.6; NVA	101.2; NVA	100.3; NVA
8	101.2; NVA	101.5; NVA	100.6; NVA	101.4; NVA	100.6; NVA
15	101.2; NVA	Moved[c]	Moved[c]	101.2; NVA	101.3; NVA
22	101.1; NVA	Moved	Moved	101.3; NVA	101.5; NVA
29	101.3; NVA	Moved	Moved	101.2; NVA	101.1; NVA

	1235	1245	1249
1	101.2; NVA	101.4; NVA	100.6; NVA
8	101.2; NVA	101.2; NVA	1-1.3; NVA
15	101.1; NVA	101.3; NVA	101.5; NVA
22	101.3; NVA	101.2; NVA	101.1; NVA
29	101.3; NVA	100.7; NVA	100.6; NVA

[a] Body temperature, °F
[b] NVA = no visible abnormalities
[c] Moved = began dosing with 5 μg 1α-Hydroxyvitamin D_5/kg on Day 9

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 4 (cont.)

Individual Animal Weekly Physical Examination Data - Females
Group 2 - Low - 10 µg/kg

Day	Animal Number		
	1242	1246	1250
-5	101.6[a]; NVA[b]	102.0; NVA	101.0; NVA
1	100.3; NVA	100.7; NVA	102.1; NVA
8	101.0; NVA	101.6; NVA	101.7; NVA
15	100.8; NVA	101.7; NVA	101.7; NVA
22	98.5; NVA	101.1; NVA	101.8; NVA
29	98.4; Thin	99.0; Thin	100.0; NVA

[a] Body temperature, °F
[b] NVA = no visible abnormalities

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 4 (cont.)

Individual Animal Weekly Physical Examination Data - Females
Group 3 - Mid - 30 µg/kg

Day	Animal Number		
	1238	1239	1243
-5	100.9[a]; NVA[b]	101.2; NVA	102.3; NVA
1	101.1; NVA	101.0; NVA	101.1; NVA
8	101.1; NVA	100.8; NVA	101.7; NVA
15	100.3; NVA	100.4; NVA	100.6; Conjunctivitis (bilateral)
22	100.6; Emaciated	98.4; Emaciated	99.3; Emaciated
29	100.9; Thin	Dead	95.1; Emaciated

[a] Body temperature, °F
[b] NVA = no visible abnormalities

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 4 (cont.)

Individual Animal Weekly Physical Examination Data - Females
Group 4 - High - 90/45[a] μg/kg

Day	Animal Number				
	1237	1240	1241	1247	1248
-5	101.7[b]; NVA[c]	102.8; NVA	102.6; NVA	102.2; NVA	101.5; NVA
1	101.3; NVA	101.7; Conjunctivitis (right eye)	100.7; Conjunctivitis (right eye)	101.3; NVA	101.0; NVA
8	101.2; NVA	100.8; NVA	101.7; Conjunctivitis (right eye)	Dead	Dead
15	100.6; NVA	100.5; NVA	101.5; Conjunctivitis (right eye)	Dead	Dead
22	100.2; Emaciated	100.5; Emaciated	101.5; Emaciated; Conjunctivitis (right eye)	Dead	Dead
29	100.0 Thin	99.1; Emaciated	101.1; Emaciated; Conjunctivitis (right eye)	Dead	Dead

[a] dose decreased from 90 to 45 μg/kg on Day 9
[b] Body temperature, °F
[c] NVA = no visible abnormalities

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 4 (cont.)

Individual Animal Weekly Physical Examination Data - Females
Group 5 - Low-Low - 5 µg/kg

Day	Animal Number	
	1236	1244
1	101.5[a]; NVA[b]	100.6; NVA
8	101.6; NVA	102.2; Conjunctivitis (bilateral)
15	101.5; NVA	102.5; NVA
22	101.5; NVA	101.8; NVA
29	100.6; NVA	100.9; NVA

[a] Body temperature, °F
[b] NVA = no visible abnormalities

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 5

Summary of Mean Body Weights (kg)

Males

Group	Dose (µg/kg)		Day 1[a]	Day 8	Day 15	Day 22	Day 29
1(VCTL)	0	MEAN	7.45	7.92	7.84	8.26	8.28
		SD	0.49	0.69	0.08	0.27	0.17
		N	5	5	3	3	3
2	10	MEAN	6.89	6.75*	6.23*	5.67*	5.03*
		SD	0.08	0.44	0.63	0.65	0.68
		N	3	3	3	3	3
3	30	MEAN	7.47	6.70*	5.73*	4.96*	4.96*
		SD	0.60	0.29	0.35	0.43	NA
		N	3	3	3	3	1
4	90/45[b]	MEAN	7.28	6.32*	5.56*	4.95*	4.71*
		SD	0.38	0.35	0.39	0.40	0.35
		N	5	5	5	5	2
1 (VCTL)	0	MEAN	7.71	7.84	8.26	8.28	8.61
		SD	0.10	0.08	0.27	0.17	0.09
		N	3	3	3	3	3
5	5	MEAN	8.22	8.23	8.59	8.71	8.58
		SD	1.24	1.29	1.06	0.98	0.57
		N	2	2	2	2	2

[a] predose
[b] dose decreased from 90 to 45 µg/kg on Day 9
* = significantly different from vehicle control, $p \leq 0.05$

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 5 (cont.)

Summary of Mean Body Weights (kg)

Females

Group	Dose (μg/kg)		Day 1[a]	Day 8	Day 15	Day 22	Day 29
1 (VCTL)	0	MEAN	6.96	7.01	7.23	7.66	8.11
		SD	0.42	0.51	0.66	0.71	0.84
		N	5	5	3	3	3
2	10	MEAN	6.51	6.14	5.80*	5.39*	4.91*
		SD	0.63	0.77	0.74	0.67	0.54
		N	3	3	3	3	3
3	30	MEAN	6.78	5.68*	4.91*	4.33*	3.84*
		SD	0.08	0.14	0.15	0.19	0.28
		N	3	3	3	3	2
4	90/45[b]	MEAN	7.08	5.67*	4.87*	4.51*	4.32*
		SD	0.42	0.21	0.04	0.16	0.33
		N	5	3	3	3	3
1 (VCTL)	0	MEAN	7.26	7.23	7.66	8.11	7.96
		SD	0.50	0.66	0.71	0.84	0.77
		N	3	3	3	3	3
5	5	MEAN	6.63	6.56	6.89	7.14	6.97
		SD	0.21	0.31	0.33	0.31	0.41
		N	2	2	2	2	2

[a] predose
[b] dose decreased from 90 to 45 μg/kg on Day 8
* = significantly different from vehicle control, $p \leq 0.05$

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 6

Summary of Mean Body Weight Gains (kg)

Males

Group	Dose (µg/kg)		Day 8	Day 15	Day 22	Day 29	Total
1 (VCTL)	0	MEAN	0.47	0.13	0.42	0.02	0.95
		SD	0.25	0.03	0.19	0.12	0.08
		N	5	3	3	3	3
2	10	MEAN	-0.14*	-0.52*	-0.56*	-0.63*	-1.85*
		SD	0.47	0.32	0.14	0.25	0.63
		N	3	3	3	3	3
3	30	MEAN	-0.77*	-0.97*	-0.77*	-0.44*	-2.72*
		SD	0.37	0.06	0.14	NA	NA
		N	3	3	3	1	1
4	90/45[a]	MEAN	-0.96*	-0.76*	-0.61*	-0.54*	-2.77*
		SD	0.14	0.06	0.23	0.00	0.13
		N	5	5	5	2	2
1 (VCTL)	0	MEAN	0.13	0.42	0.02	0.33	0.89
		SD	0.03	0.19	0.13	0.08	0.03
		N	3	3	3	3	3
5	5	MEAN	0.01*	0.36	0.12	-0.13	0.36
		SD	0.04	0.23	0.09	0.41	0.68
		N	2	2	2	2	2

[a] dose decreased from 90 to 45 µg/kg on Day 9
* = significantly different from vehicle control, $p \leq 0.05$

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 6 (cont.)

Summary of Mean Body Weight Gains (kg)

Females

Group	Dose (μg/kg)		Day 8	Day 15	Day 22	Day 29	Total
1 (VCTL)	0	MEAN	0.05	0.17	0.36	0.33	0.95
		SD	0.21	0.30	0.12	0.36	0.62
		N	5	3	3	3	3
2	10	MEAN	-0.37*	-0.34*	-0.41*	-0.49*	-1.61*
		SD	0.22	0.05	0.12	0.15	0.12
		N	3	3	3	3	3
3	30	MEAN	-1.10*	-0.77*	-0.57*	-0.51*	-2.94*
		SD	0.11	0.02	0.10	0.01	0.17
		N	3	3	3	2	3
4	90/45[a]	MEAN	-1.31*	-0.80*	-0.36*	-0.19	-2.67*
		SD	0.01	0.21	0.13	0.19	0.43
		N	3	3	3	3	3
1 (VCTL)	0	MEAN	-0.03	0.43	0.45	-0.15	0.70
		SD	0.16	0.11	0.15	0.07	0.28
		N	3	3	3	3	3
5	5	MEAN	-0.07	0.33	0.25	-0.17	0.34
		SD	0.10	0.01	0.01	0.10	0.20
		N	2	2	2	2	2

[a] dose decreased from 90 to 45 μg/kg on Day 8
* = significantly different from vehicle control, $p \leq 0.05$

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 7

Summary of Mean Daily Food Consumption (g)

Males

Group	Dose (µg/kg)		Day 1	2	3	4	5	6	7	8	9
1 (VCTL)	0	MEAN	221	166	201	201	241	220	228	233	155
		SD	98.3	48.7	22.6	40.0	34.3	58.6	81.7	82.5	12.7
		N	5	5	5	5	5	5	5	5	3
2	10	MEAN	163	171	106*	158	166	135*	180	222	73*
		SD	135.5	58.3	51.6	41.5	70.1	48.9	105.8	83.4	77.1
		N	3	3	3	3	3	3	3	3	3
3	30	MEAN	215	148	164	170	86*	93*	80*	29*	0*
		SD	58.4	10.0	3.8	53.5	25.9	32.0	33.9	27.7	0.6
		N	3	3	3	3	3	3	3	3	3
4	90/45[a]	MEAN	244	122	95*	84*	16*	9*	35*	7*	28*
		SD	58.0	9.8	34.1	25.2	26.1	12.8	39.5	10.3	12.2
		N	5	5	5	5	5	5	5	5	5
1 (VCTL)	0	MEAN	155	193	223	187	265	269	271	248	228
		SD	12.7	16.0	13.5	29.5	18.2	13.4	37.4	45.4	7.2
		N	3	3	3	3	3	3	3	3	3
5	5	MEAN	178	255*	214	202	278	274	266	296	241
		SD	74.2	2.1	43.8	29.0	31.1	37.5	37.5	5.7	58.0
		N	2	2	2	2	2	2	2	2	2

[a] dose decreased from 90 to 45 µg/kg on Day 9
* = significantly different from vehicle control, p≤ 0.05

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 7 (cont.)

Summary of Mean Daily Food Consumption (g) - Males (cont.)

Group	Dose (µg/kg)		Day 10	11	12	13	14	15	16	17	18
1 (VCTL)	0	MEAN	193	223	187	265	269	271	248	228	298
		SD	16.0	13.5	29.5	18.2	13.4	37.4	45.4	7.2	4.0
		N	3	3	3	3	3	3	3	3	3
2	10	MEAN	89*	127	73*	99*	108*	82*	70*	55*	70*
		SD	70.3	102.8	56.9	112.5	96.0	7.6	62.8	63.1	44.4
		N	3	3	3	3	3	3	3	3	3
3	30	MEAN	31*	8*	1*	0*	6*	10*	5*	16*	11*
		SD	10.4	14.4	2.3	0.0	5.5	8.4	8.1	17.6	7.1
		N	3	3	3	3	3	3	3	3	3
4	90/45[a]	MEAN	12*	13*	4*	28*	28*	31*	30*	38*	26*
		SD	12.6	18.6	7.8	25.5	25.9	31.2	19.9	39.2	10.1
		N	5	5	5	5	5	5	5	5	5
1 (VCTL)	0	MEAN	298	294	298	300	300	250	278	214	300
		SD	4.0	9.8	3.5	0.0	0.0	33.8	27.5	74.4	0.0
		N	3	3	3	3	3	3	3	3	3
5	5	MEAN	291	300	298	300	297	251	264	230	300
		SD	12.7	0.0	3.5	0.0	4.2	69.3	50.9	16.3	0.0
		N	2	2	2	2	2	2	2	2	2

[a] dose decreased from 90 to 45 µg/kg on Day 9

* = significantly different from vehicle control, $p \leq 0.05$

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 7 (cont.)

Summary of Mean Daily Food Consumption (g) - Males (cont.)

Group	Dose (µg/kg)		Day 19	20	21	22	23	24	25	26	27	28
1 (VCTL)	0	MEAN	294	298	300	300	250	278	214	300	280	299
		SD	9.8	3.5	0.0	0.0	33.8	27.5	74.4	0.0	35.2	2.3
		N	3	3	3	3	3	3	3	3	3	3
2	10	MEAN	70*	54*	72*	68*	41*	39*	32*	67*	35*	83*
		SD	48.5	23.1	23.9	45.1	32.3	15.0	20.1	30.2	29.2	23.8
		N	3	3	3	3	3	3	3	3	3	3
3	30	MEAN	14*	11*	16*	18*	0*	28*	2*	19*	2*	6*
		SD	12.4	9.5	14.4	30.0	0.0	NA	NA	NA	NA	NA
		N	3	3	3	3	3	1	1	1	1	1
4	90/45[a]	MEAN	43*	49*	57*	14*	27*	38*	19*	57*	23*	89*
		SD	37.2	48.2	36.2	16.8	41.1	49.3	12.2	86.1	32.5	77.1
		N	5	5	5	5	3	3	3	3	2	2
1 (VCTL)	0	MEAN	280	299	300	281	300	300	138	183	300	300
		SD	35.2	2.3	0.0	32.3	0.0	0.0	21.9	5.7	0.0	0.0
		N	3	3	3	3	3	3	3	3	3	3
5	5	MEAN	295	300	300	285	300	300	124	191	300	300
		SD	7.1	0.0	0.0	10.6	0.0	0.0	80.6	51.6	0.0	0.0
		N	2	2	2	2	2	2	2	2	2	2

[a] dose decreased from 90 to 45 µg/kg on Day 9
* = significantly different from vehicle control, $p \leq 0.05$

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 7 (cont.)

Summary of Mean Daily Food Consumption (g) - Females

Group	Dose (µg/kg)		Day 1	2	3	4	5	6	7	8	9
1 (VCTL)	0	MEAN	130	148	158	212	174	212	233	184	187
		SD	61.2	48.5	16.9	28.2	32.5	76.2	94.4	59.9	26.8
		N	5	5	5	5	5	5	5	3	3
2	10	MEAN	143	124	102*	174	119	172	233	96*	117*
		SD	43.3	40.5	11.0	34.0	21.6	23.1	59.2	11.6	41.6
		N	3	3	3	3	3	3	3	3	3
3	30	MEAN	98	106	90*	73*	63*	64*	58*	7*	6*
		SD	85.4	42.5	33.5	28.4	40.2	27.8	35.0	6.6	7.2
		N	3	3	3	3	3	3	3	3	3
4	90/45[a]	MEAN	119	130	66*	68*	16*	14*	0*	13*	9*
		SD	42.6	30.2	28.9	56.6	30.9	17.3	0.0	11.5	12.3
		N	5	5	5	5	5	4	3	3	3
1 (VCTL)	0	MEAN	184	187	231	229	263	250	188	248	219
		SD	59.9	26.8	59.8	47.3	32.4	35.3	31.1	25.7	38.4
		N	3	3	3	3	3	3	3	3	3
5	5	MEAN	119	189	253	217	300	291	179	266	205
		SD	77.8	14.8	67.2	31.8	0.0	12.7	16.3	36.8	19.8
		N	2	2	2	2	2	2	2	2	2

[a] dose decreased from 90 to 45 µg/kg on Day 8
* = significantly different from vehicle control, p≤ 0.05

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 7 (cont.)

Summary of Mean Daily Food Consumption (g) - Females (cont.)

Group	Dose (μg/kg)		Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18
1 (VCTL)	0	MEAN	231	229	263	250	188	248	219	269	257
		SD	59.8	47.3	32.4	35.3	31.1	25.7	38.4	27.2	38.4
		N	3	3	3	3	3	3	3	3	3
2	10	MEAN	104*	126*	148*	139*	125	119*	81*	104*	94*
		SD	29.1	17.6	42.4	35.5	48.4	66.9	30.1	27.2	66.0
		N	3	3	3	3	3	3	3	3	3
3	30	MEAN	3*	4*	7*	15*	6*	10*	10*	18*	14*
		SD	3.2	6.7	6.5	7.8	5.0	9.5	3.8	15.3	14.5
		N	3	3	3	3	3	3	3	3	3
4	90/45[a]	MEAN	7*	15*	13*	20*	21*	11*	24*	24*	33*
		SD	11.3	20.4	6.4	6.4	21.8	6.0	18.0	15.9	28.0
		N	3	3	2	2	3	3	3	3	3
1 (VCTL)	0	MEAN	269	257	283	300	300	242	247	203	300
		SD	27.2	38.4	22.7	0.0	0.0	52.0	48.3	89.0	0.0
		N	3	3	3	3	3	3	3	3	3
5	5	MEAN	298	269	300	300	300	197	238	134	300
		SD	2.8	29.0	0.0	0.0	0.0	22.6	13.4	28.3	0.0
		N	2	2	2	2	2	2	2	2	2

[a] dose decreased from 90 to 45 μg/kg on Day 8
* = significantly different from vehicle control, p≤ 0.05

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 7 (cont.)

Summary of Mean Daily Food Consumption (g) - Females (cont.)

Group	Dose (µg/kg)		Day 19	20	21	22	23	24	25	26	27	28
1 (VCTL)	0	MEAN	283	300	300	242	247	203	300	285	300	300
		SD	22.7	0.0	0.0	52.0	48.3	89.0	0.0	25.4	0.0	0.0
		N	3	3	3	3	3	3	3	3	3	3
2	10	MEAN	80*	111*	49*	16*	39*	45*	37*	37*	30*	22*
		SD	32.3	8.5	12.5	5.0	11.0	21.0	4.7	7.2	24.1	7.6
		N	3	3	3	3	3	3	3	3	3	3
3	30	MEAN	20*	30*	9*	8*	10*	16*	32*	9*	9*	1*
		SD	13.0	20.6	5.6	6.7	4.0	17.9	27.5	9.5	3.5	1.4
		N	3	3	3	3	3	3	3	3	3	2
4	90/45[a]	MEAN	29*	53*	18*	53*	73*	24*	89*	22*	64*	65*
		SD	4.6	54.0	12.1	36.2	50.9	17.8	77.1	32.0	84.0	49.4
		N	3	3	3	3	3	3	3	3	3	3
1 (VCTL)	0	MEAN	285	300	300	277	300	300	218	210	300	300
		SD	25.4	0.0	0.0	40.4	0.0	0.0	141.5	87.6	0.0	0.0
		N	3	3	3	3	3	3	3	3	3	3
5	5	MEAN	267	248	300	265	300	300	45	138	300	300
		SD	47.4	43.8	0.0	50.2	0.0	0.0	37.5	8.5	0.0	0.0
		N	2	2	2	2	2	2	2	2	2	2

[a] dose decreased from 90 to 45 µg/kg on Day 8

* = significantly different from vehicle control, p≤ 0.05

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 8

Summary of Mean Hematology Data - Males

Pre-test

Group	Dose (μg/kg)		WBC thsn/cmm	RBC mill/cmm	HGB g/dL	HCT %	MCV fl	MCH pg	MCHC %	PLT thsn/cmm	RETPC %
1 (VCTL)	0	MEAN	14.5	6.67	15.0	45.0	67.4	22.5	33.4	440	1.5
		SD	5.91	0.182	0.48	1.46	1.46	0.55	0.32	51.9	0.47
		N	5	5	5	5	5	5	5	5	5
2	10	MEAN	16.4	6.46	14.7	43.7	67.9	22.8	33.6	408	1.4
		SD	5.09	0.764	1.11	3.60	2.80	1.12	0.29	29.6	0.82
		N	3	3	3	3	3	3	3	3	3
3	30	MEAN	15.1	6.74	15.6	47.2	70.0	23.1	33.0	415	2.7*
		SD	4.75	0.195	1.00	2.73	3.71	1.31	0.25	60.6	0.45
		N	3	3	3	3	3	3	3	3	3
4	90	MEAN	14.2	6.51	14.9	45.0	69.1	22.8	33.0	443	1.7
		SD	2.76	0.368	0.84	2.47	2.34	0.86	0.26	74.1	0.57
		N	5	5	5	5	5	5	5	5	5

* significantly different from vehicle control, p ≤ 0.05

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 8 (cont.)

Summary of Mean Hematology Data - Males

Pre-test

Group	Dose (μg/kg)		RETABS thsn/cmm	NRBC #/100 WBC	SEG NEU thsn/cmm	BAND NEU thsn/cmm	LYMPH thsn/cmm	MONO thsn/cmm	EOSIN thsn/cmm	BASO thsn/cmm
1 (VCTL)	0	MEAN	99.5	0.0	10.2	0.4	2.9	0.8	0.2	0.0
		SD	29.79	0.00	4.77	0.49	1.15	0.38	0.22	0.00
		N	5	5	5	5	5	5	5	5
2	10	MEAN	92.0	0.0	12.1	0.4	3.1	0.6	0.2	0.0
		SD	65.82	0.00	3.78	0.20	0.79	0.44	0.15	0.00
		N	3	3	3	3	3	3	3	3
3	30	MEAN	184.4*	0.0	10.5	0.1	3.4	0.8	0.1	0.0
		SD	32.38	0.00	3.50	0.10	1.47	0.45	0.12	0.00
		N	3	3	3	3	3	3	3	3
4	90	MEAN	107.1	0.2	10.3	0.2	2.8	0.9	0.2	0.0
		SD	32.81	0.45	2.35	0.18	0.68	0.39	0.08	0.00
		N	5	5	5	5	5	5	5	5

* significantly different from vehicle control, $p \leq 0.05$

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 8 (cont.)

Summary of Mean Hematology Data - Males

Pre-test

Group	Dose (µg/kg)		NRBC #/100 WBC	SEG NEU %	BAND NEU %	LYMPH %	MONO %	EOSIN %	BASO %
1 (VCTL)	0	**MEAN**	0	71	2	20	5	2	0
		SD	0.0	7.8	2.0	6.8	2.1	1.6	0.0
		N	5	5	5	5	5	5	5
2	10	**MEAN**	0	74	3	19	3	1	0
		SD	0.0	1.0	1.2	1.0	1.5	1.0	0.0
		N	3	3	3	3	3	3	3
3	30	**MEAN**	0	70	1	22	6	1	0
		SD	0.0	6.0	0.6	2.3	4.9	1.0	0.0
		N	3	3	3	3	3	3	3
4	90	**MEAN**	0	72	1	20	6	1	0
		SD	0.4	4.5	1.3	5.2	1.8	0.4	0.0
		N	5	5	5	5	5	5	5

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 8 (cont.)

Summary of Mean Hematology Data - Females

Pre-test

Group	Dose (µg/kg)		WBC thsn/cmm	RBC mill/cmm	HGB g/dL	HCT %	MCV fl	MCH pg	MCHC %	PLT thsn/cmm	RETPC %
1 (VCTL)	0	MEAN	10.7	6.86	15.7	46.9	68.3	22.9	33.5	439	1.7
		SD	1.65	0.375	1.12	3.76	4.16	1.37	0.59	48.5	0.50
		N	5	5	5	5	5	5	5	5	5
2	10	MEAN	13.6	6.66	15.5	46.4	69.6	23.3	33.4	366	2.2
		SD	2.86	0.334	0.92	2.25	0.50	0.20	0.49	22.9	0.49
		N	3	3	3	3	3	3	3	3	3
3	30	MEAN	10.1	6.46	15.1	44.8	69.2	23.3	33.7	376	1.6
		SD	2.66	0.666	1.36	4.82	0.56	0.51	0.97	8.5	0.62
		N	3	3	3	3	3	3	3	3	3
4	90	MEAN	14.0	6.62	15.3	45.2	68.3	23.1	33.8	454	1.3
		SD	1.02	0.279	0.23	0.90	1.97	0.73	0.42	104.1	0.46
		N	5	5	5	5	5	5	5	5	5

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 8 (cont.)

Summary of Mean Hematology Data - Females

Pre-test

Group	Dose (µg/kg)		RETABS thsn/cmm	NRBC #/100 WBC	SEG NEU thsn/cmm	BAND NEU thsn/cmm	LYMPH thsn/cmm	MONO thsn/cmm	EOSIN thsn/cmm	BASO thsn/cmm
1 (VCTL)	0	MEAN	119.9	0.2	6.9	0.1	3.1	0.5	0.1	0.0
		SD	35.73	0.45	1.82	0.12	0.44	0.33	0.07	0.00
		N	5	5	5	5	5	5	5	5
2	10	MEAN	145.4	0.0	8.9	0.3	3.4	0.9	0.1	0.0
		SD	39.19	0.00	2.59	0.40	0.72	0.45	0.10	0.00
		N	3	3	3	3	3	3	3	3
3	30	MEAN	102.6	0.0	6.8	0.2	2.5	0.5	0.2	0.0
		SD	38.36	0.00	2.35	0.15	0.36	0.06	0.06	0.00
		N	3	3	3	3	3	3	3	3
4	90	MEAN	84.0	0.0	9.8	0.0	3.3	0.7	0.2	0.0
		SD	32.59	0.00	0.82	0.05	0.70	0.19	0.13	0.00
		N	5	5	5	5	5	5	5	5

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 8 (cont.)

Summary of Mean Hematology Data - Females

Pre-test

Group	Dose (µg/kg)		NRBC #/100 WBC	SEG NEU %	BAND NEU %	LYMPH %	MONO %	EOSIN %	BASO %
1 (VCTL)	0	MEAN	0	64	1	29	5	1	0
		SD	0.4	8.3	0.8	6.1	3.4	0.7	0.0
		N	5	5	5	5	5	5	5
2	10	MEAN	0	65	2	26	6	1	0
		SD	0.0	6.7	2.3	9.3	2.1	1.0	0.0
		N	3	3	3	3	3	3	3
3	30	MEAN	0	67	2	25	5	2	0
		SD	0.0	5.5	1.0	4.4	1.5	0.6	0.0
		N	3	3	3	3	3	3	3
4	90	MEAN	0	70	0	24	5	1	0
		SD	0.0	4.1	0.4	4.5	1.1	0.8	0.0
		N	5	5	5	5	5	5	5

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 9

Summary of Mean Hematology Data - Males

Post-dose

Group	Dose (µg/kg)		WBC thsn/cmm	RBC mill/cmm	HGB g/dL	HCT %	MCV fl	MCH pg	MCHC %	PLT thsn/cmm	RETPC %
1 (VCTL)	0	MEAN	12.8	6.36	14.2	42.7	67.1	22.3	33.2	340	1.2
		SD	5.10	0.406	0.86	2.98	0.82	0.15	0.32	22.7	0.21
		N	3	3	3	3	3	3	3	3	3
2	10	MEAN	9.7	8.34	18.3*	56.2	67.7	22.1	32.6	303	0.8
		SD	2.36	1.310	1.60	6.66	2.78	1.55	1.08	72.0	0.21
		N	3	3	3	3	3	3	3	3	3
3	30	MEAN	17.7	9.03	19.8*	61.2*	68.0	22.0	32.4	364	0.9
		SD	5.52	1.485	2.26	7.50	2.83	1.13	0.28	84.9	0.50
		N	2	2	2	2	2	2	2	2	2
4	90/45[a]	MEAN	12.4	8.70*	20.1*	61.0*	70.2	23.1	32.9	303	0.1*
		SD	5.34	1.017	1.93	5.92	1.65	0.51	0.53	124.7	0.10
		N	4	4	4	4	4	4	4	4	4
1 (VCTL)	0	MEAN	15.4	6.68	15.0	45.0	67.4	22.4	33.2	339	1.2
		SD	4.31	0.047	0.35	0.71	1.23	0.59	0.29	24.8	0.38
		N	3	3	3	3	3	3	3	3	3
5	5	MEAN	19.7	6.74	15.3	45.5	67.5	22.7	33.6	259	1.2
		SD	14.00	0.318	0.99	3.61	2.19	0.42	0.50	75.0	0.42
		N	2	2	2	2	2	2	2	2	2

[a] dose decreased from 90 to 45 µg/kg on Day 9
* significantly different from vehicle control, $p \leq 0.05$

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 9 (cont.)

Summary of Mean Hematology Data - Males

Post-dose

Group	Dose (µg/kg)		RETABS thsn/cmm	NRBC #/100 WBC	SEG NEU thsn/cmm	BAND NEU thsn/cmm	LYMPH thsn/cmm	MONO thsn/cmm	EOSIN thsn/cmm	BASO thsn/cmm
1 (VCTL)	0	MEAN	73.7	0.0	9.1	0.4	2.5	0.5	0.3	0.0
		SD	8.65	0.00	3.70	0.20	1.12	0.27	0.30	0.00
		N	3	3	3	3	3	3	3	3
2	10	MEAN	70.8	0.0	7.5	0.1	1.6	0.4	0.0	0.0
		SD	24.83	0.00	2.63	0.06	0.44	0.10	0.00	0.00
		N	3	3	3	3	3	3	3	3
3	30	MEAN	73.1	0.0	10.4	3.3	2.3	1.7	0.1	0.0
		SD	32.10	0.00	0.28	4.53	0.78	1.34	0.14	0.00
		N	2	2	2	2	2	2	2	2
4	90/45[a]	MEAN	6.1*	0.0	8.8	0.9	1.8	0.9	0.1	0.0
		SD	7.83	0.00	4.52	0.33	0.33	0.61	0.10	0.00
		N	4	4	4	4	4	4	4	4
1 (VCTL)	0	MEAN	78.0	0.0	10.6	0.1	3.5	0.8	0.3	0.0
		SD	25.54	0.00	3.69	0.10	0.97	0.35	0.31	0.00
		N	3	3	3	3	3	3	3	3
5	5	MEAN	81.5	0.5	11.0	3.3	4.1	1.3	0.1	0.0
		SD	32.39	0.71	7.07	4.60	0.92	1.56	0.14	0.00
		N	2	2	2	2	2	2	2	2

[a] dose decreased from 90 to 45 µg/kg on Day 9
* significantly different from vehicle control, $p \leq 0.05$

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 9 (cont.)

Summary of Mean Hematology Data - Males

Post-dose

Group	Dose (μg/kg)		NRBC #/100 WBC	SEG NEU %	BAND NEU %	LYMPH %	MONO %	EOSIN %	BASO %
1 (VCTL)	0	MEAN	0	72	3	20	4	2	0
		SD	0.0	7.5	1.0	5.0	2.1	1.7	0.0
		N	3	3	3	3	3	3	3
2	10	MEAN	0	76	2	18	4	0	0
		SD	0.0	9.9	0.6	9.5	1.0	0.0	0.0
		N	3	3	3	3	3	3	3
3	30	MEAN	0	62	16	14	9	1	0
		SD	0.0	17.7	20.5	8.5	5.0	0.7	0.0
		N	2	2	2	2	2	2	2
4	90/45[a]	MEAN	0	69	8	17	6	0	0
		SD	0.0	9.3	3.9	6.7	4.2	0.5	0.0
		N	4	4	4	4	4	4	4
1 (VCTL)	0	MEAN	0	68	1	24	5	2	0
		SD	0.0	6.1	0.6	6.1	2.1	2.0	0.0
		N	3	3	3	3	3	3	3
5	5	MEAN	1	58	11	26	5	1	0
		SD	0.7	5.0	15.6	13.4	4.2	1.4	0.0
		N	2	2	2	2	2	2	2

[a] dose decreased from 90 to 45 μg/kg on Day 9
* significantly different from vehicle control, $p \leq 0.05$

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 9 (cont.)

Summary of Mean Hematology Data - Females

Post-dose

Group	Dose (μg/kg)	Group	WBC thsn/cmm	RBC mill/cmm	HGB g/dL	HCT %	MCV fl	MCH pg	MCHC %	PLT thsn/cmm	RETPC %
1 (VCTL)	0	MEAN	9.4	6.53	14.9	44.4	68.1	22.8	33.5	342	1.2
		SD	0.83	0.356	1.47	4.10	5.12	1.82	0.23	24.9	0.25
		N	3	3	3	3	3	3	3	3	3
2	10	MEAN	7.6	7.68	17.8*	54.0*	70.3	23.1	32.9	310	0.7
		SD	1.32	0.214	0.55	2.21	0.95	0.21	0.49	41.3	0.15
		N	3	3	3	3	3	3	3	3	3
3	30	MEAN	8.7	8.87*	20.2*	61.0*	68.9	22.8	33.2	399	0.5
		SD	2.17	0.255	0.23	0.97	1.19	0.49	0.21	53.3	0.31
		N	3	3	3	3	3	3	3	3	3
4	90/45[a]	MEAN	11.0	8.09*	19.0*	56.4*	69.8	23.5	33.6	379	0.6
		SD	2.67	0.857	1.00	4.19	2.11	1.17	0.72	87.8	0.57
		N	3	3	3	3	3	3	3	3	3
1 (VCTL)	0	MEAN	14.8	6.69	15.2	45.8	68.4	22.7	33.2	385	1.7
		SD	0.15	0.315	1.31	3.71	4.24	1.34	0.45	48.5	0.66
		N	3	3	3	3	3	3	3	3	3
5	5	MEAN	13.2	7.06	16.3	48.5	68.8	23.0	33.5	345	1.1
		SD	1.34	0.544	1.06	3.11	0.92	0.28	0.00	9.9	0.35
		N	2	2	2	2	2	2	2	2	2

[a] dose decreased from 90 to 45 μg/kg on Day 8
* significantly different from vehicle control, $p \leq 0.05$

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 9 (cont.)

Summary of Mean Hematology Data - Females

Post-dose

Group	Dose (µg/kg)	Group	RETABS thsn/cmm	NRBC #/100 WBC	SEG NEU thsn/cmm	BAND NEU thsn/cmm	LYMPH thsn/cmm	MONO thsn/cmm	EOSIN thsn/cmm	BASO thsn/cmm
1 (VCTL)	0	MEAN	76.7	0.0	6.4	0.1	2.6	0.3	0.0	0.0
		SD	20.02	0.00	1.23	0.10	0.53	0.06	0.06	0.00
		N	3	3	3	3	3	3	3	3
2	10	MEAN	56.3	0.0	4.3	0.2	2.3	0.5	0.2*	0.0
		SD	11.93	0.00	1.22	0.40	0.86	0.47	0.06	0.00
		N	3	3	3	3	3	3	3	3
3	30	MEAN	41.9	0.0	6.3	0.0	1.9	0.4	0.0	0.0
		SD	28.19	0.00	1.61	0.06	1.08	0.15	0.06	0.00
		N	3	3	3	3	3	3	3	3
4	90/45[a]	MEAN	49.1	0.0	7.0	0.3	2.6	1.0	0.0	0.0
		SD	53.02	0.00	3.57	0.35	0.70	0.30	0.06	0.00
		N	3	3	3	3	3	3	3	3
1 (VCTL)	0	MEAN	112.4	0.3	8.4	0.3	4.6	1.0	0.5	0.0
		SD	39.72	0.58	0.87	0.30	0.31	0.58	0.44	0.00
		N	3	3	3	3	3	3	3	3
5	5	MEAN	73.1	0.0	8.7	0.3	3.1	0.8	0.5	0.0
		SD	19.23	0.00	1.34	0.35	1.06	0.50	0.21	0.00
		N	2	2	2	2	2	2	2	2

[a] dose decreased from 90 to 45 µg/kg on Day 8
* significantly different from vehicle control, $p \leq 0.05$

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 9 (cont.)

Summary of Mean Hematology Data - Females

Post-dose

Group	Dose (μg/kg)		NRBC #/100 WBC	SEG NEU %	BAND NEU %	LYMPH %	MONO %	EOSIN %	BASO %
1 (VCTL)	0	MEAN	0	67	1	28	4	0	0
		SD	0.0	8.5	1.0	7.0	0.6	0.6	0.0
		N	3	3	3	3	3	3	3
2	10	MEAN	0	56	3	33	6	3*	0
		SD	0.0	6.6	4.6	14.6	4.7	1.0	0.0
		N	3	3	3	3	3	3	3
3	30	MEAN	0	73	0	21	5	0	0
		SD	0.0	10.8	0.6	8.7	1.7	0.6	0.0
		N	3	3	3	3	3	3	3
4	90/45[a]	MEAN	0	61	3	26	10	0	0
		SD	0.0	15.9	3.5	11.0	4.6	0.6	0.0
		N	3	3	3	3	3	3	3
1 (VCTL)	0	MEAN	0	57	2	31	7	4	0
		SD	0.6	6.4	2.0	2.0	3.5	2.9	0.0
		N	3	3	3	3	3	3	3
5	5	MEAN	0	66	2	23	6	4	0
		SD	0.0	3.5	2.8	5.7	4.2	2.1	0.0
		N	2	2	2	2	2	2	2

[a] dose decreased from 90 to 45 μg/kg on Day 8
* significantly different from vehicle control, $p \leq 0.05$

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 10

Summary of Mean Coagulation Data - Males

Pre-test

Group	Dose (μg/kg)	Group	PT sec	APTT sec	FIB mg/dL
1 (VCTL)	0	MEAN	8.7	11.1	185
		SD	0.12	0.68	34.8
		N	5	5	5
2	10	MEAN	8.6	11.6	203
		SD	0.10	1.53	23.8
		N	3	3	3
3	30	MEAN	8.5	10.1	107
		SD	0.15	0.40	27.7
		N	3	3	3
4	90	MEAN	10.2	10.9	208
		SD	3.38	0.78	30.2
		N	5	5	5

* significantly different from vehicle control, $p \leq 0.05$

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 10 (cont.)

Summary of Mean Coagulation Data - Females

Pre-test

Group	Dose (µg/kg)	Group	PT sec	APTT sec	FIB mg/dL
1 (VCTL)	0	MEAN	8.9	10.8	161
		SD	0.22	0.64	17.8
		N	5	5	5
2	10	MEAN	8.8	11.5	178
		SD	0.21	0.75	56.8
		N	3	3	3
3	30	MEAN	8.7	11.0	146
		SD	0.06	0.91	12.3
		N	3	3	3
4	90	MEAN	8.8	11.5	190
		SD	0.27	0.82	29.6
		N	5	5	5

* significantly different from vehicle control, $p \leq 0.05$

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 11

Summary of Mean Coagulation Data - Males

Post-dose

Group	Dose (μg/kg)	Group	PT sec	APTT sec	FIB mg/dL
1 (VCTL)	0	MEAN	7.7	10.1	180
		SD	0.15	0.23	12.2
		N	3	3	3
2	10	MEAN	7.4	12.9	347
		SD	0.21	1.29	133.8
		N	3	3	3
3	30	MEAN	7.4	14.1	416
		SD	0.00	1.20	203.6
		N	2	2	2
4	90/45[a]	MEAN	10.5	37.6	317
		SD	3.36	45.68	64.6
		N	4	4	4
1 (VCTL)	0	MEAN	7.8	10.3	182
		SD	0.23	0.44	46.6
		N	3	3	3
5	5	MEAN	8.0	10.9	275
		SD	0.07	0.71	9.2
		N	2	2	2

[a] dose decreased from 90 to 45 μg/kg on Day 9
* significantly different from vehicle control, $p \leq 0.05$

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 11 (cont.)

Summary of Mean Coagulation Data - Females

Post-dose

Group	Dose (μg/kg)	Group	PT sec	APTT sec	FIB mg/dL
1 (VCTL)	0	MEAN	7.7	9.8	170
		SD	0.15	0.32	15.5
		N	3	3	3
2	10	MEAN	7.5	11.5	238
		SD	0.06	1.12	30.4
		N	3	3	3
3	30	MEAN	7.6	14.5*	198
		SD	0.31	1.00	40.4
		N	3	3	3
4	90/45[a]	MEAN	7.3	13.6*	287*
		SD	0.12	0.49	47.0
		N	3	3	3
1 (VCTL)	0	MEAN	7.7	10.1	155
		SD	0.00	0.35	8.4
		N	3	3	3
5	5	MEAN	7.9	10.8	182*
		SD	0.21	0.50	5.7
		N	2	2	2

[a] dose decreased from 90 to 45 μg/kg on Day 8
* significantly different from vehicle control, $p \leq 0.05$

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 12

Summary of Mean Clinical Chemistry Data - Males

Pre-test

Group	Dose (μg/kg)		NA mmol/L	K mmol/L	CL mmol/L	CA mg/dL	PO4 mg/dL	ALP IU/L	ALT IU/L	AST IU/L	GGT IU/L	LDH IU/L
1 (VCTL)	0	**MEAN**	146	4.8	111	11.3	7.6	111	26	32	3	178
		SD	1.2	0.34	1.6	0.43	0.49	14.7	4.4	4.9	0.8	41.0
		N	5	5	5	5	5	5	5	5	5	5
2	10	**MEAN**	145	4.8	112	10.9	7.4	123	41	30	3	118
		SD	1.2	0.17	0.6	0.21	0.87	21.8	9.0	3.0	1.5	13.2
		N	3	3	3	3	3	3	3	3	3	3
3	30	**MEAN**	146	4.9	110	11.7	7.9	96	30	34	4	148
		SD	1.2	0.49	3.6	0.25	1.02	7.0	6.8	0.6	0.6	70.7
		N	3	3	3	3	3	3	3	3	3	3
4	90	**MEAN**	145	5.1	110	11.3	7.5	114	33	33	3	199
		SD	0.8	0.51	1.6	0.54	0.52	29.0	8.8	8.2	1.4	113.6
		N	5	5	5	5	5	5	5	5	5	5

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 12 (cont.)

Summary of Mean Clinical Chemistry Data - Males

Pre-test

Group	Dose (µg/kg)		TBIL mg/dL	BUN mg/dL	CREA mg/dL	GLU mg/dL	TP g/dL	ALB g/dL	GLOB g/dL	A/G -	CHOL mg/dL	TG mg/dL
1 (VCTL)	0	**MEAN**	0.37	11	0.7	101	5.4	3.2	2.2	1.5	150	21
		SD	0.059	1.1	0.13	8.7	0.19	0.11	0.21	0.19	20.3	6.1
		N	5	5	5	5	5	5	5	5	5	5
2	10	**MEAN**	0.43	12	0.8	93	5.1	3.1	2.0	1.6	133	25
		SD	0.136	3.0	0.12	15.3	0.15	0.06	0.20	0.15	32.1	5.7
		N	3	3	3	3	3	3	3	3	3	3
3	30	**MEAN**	0.27	14	0.7	103	5.5	3.4	2.2	1.6	144	24
		SD	0.096	3.6	0.12	8.7	0.32	0.06	0.38	0.27	6.0	7.6
		N	3	3	3	3	3	3	3	3	3	3
4	90	**MEAN**	0.34	12	0.7	102	5.5	3.3	2.2	1.5	144	22
		SD	0.130	2.3	0.15	10.1	0.39	0.22	0.36	0.27	14.7	9.5
		N	5	5	5	5	5	5	5	5	5	5

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 12 (cont.)

Summary of Mean Clinical Chemistry Data - Females

Pre-test

Group	Dose (µg/kg)		NA mmol/L	K mmol/L	CL mmol/L	CA mg/dL	PO4 mg/dL	ALP IU/L	ALT IU/L	AST IU/L	GGT IU/L	LDH IU/L
1 (VCTL)	0	MEAN	146	4.8	111	11.3	6.9	104	37	32	4	203
		SD	1.7	0.21	1.5	0.16	0.59	15.9	7.2	2.3	1.5	94.6
		N	5	5	5	5	5	5	5	5	5	5
2	10	MEAN	146	4.6	111	11.3	6.9	93	28	31	3	234
		SD	0.6	0.15	1.5	0.10	0.84	18.2	7.4	7.0	0.0	149.3
		N	3	3	3	3	3	3	3	3	3	3
3	30	MEAN	147	5.2	110	11.2	7.0	105	39	28	4	174
		SD	2.0	0.17	1.2	0.06	0.31	22.9	3.5	5.9	1.0	61.5
		N	3	3	3	3	3	3	3	3	3	3
4	90	MEAN	146	4.8	110	11.3	6.9	95	31	33	4	232
		SD	1.1	0.36	1.5	0.22	0.69	28.5	2.5	5.5	0.4	165.6
		N	5	5	5	5	5	5	5	5	5	5

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 12 (cont.)

Summary of Mean Clinical Chemistry Data - Females

Pre-test

Group	Dose (µg/kg)		TBIL mg/dL	BUN mg/dL	CREA mg/dL	GLU mg/dL	TP g/dL	ALB g/dL	GLOB g/dL	A/G -	CHOL mg/dL	TG mg/dL
1 (VCTL)	0	MEAN	0.40	11	0.7	95	5.3	3.3	2.0	1.7	141	21
		SD	0.081	1.3	0.07	4.4	0.26	0.14	0.12	0.06	30.0	4.0
		N	5	5	5	5	5	5	5	5	5	5
2	10	MEAN	0.41	12	0.8	96	5.3	3.3	2.0	1.6	137	19
		SD	0.046	1.5	0.06	2.1	0.10	0.10	0.10	0.12	5.1	2.6
		N	3	3	3	3	3	3	3	3	3	3
3	30	MEAN	0.29	12	0.7	93	5.3	3.3	2.0	1.7	138	22
		SD	0.032	3.0	0.00	4.2	0.15	0.21	0.17	0.21	24.0	6.8
		N	3	3	3	3	3	3	3	3	3	3
4	90	MEAN	0.42	13	0.7	94	5.4	3.2	2.2	1.5	146	21
		SD	0.207	1.5	0.11	7.3	0.16	0.20	0.15	0.22	16.8	4.4
		N	5	5	5	5	5	5	5	5	5	5

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 13

Summary of Mean Clinical Chemistry Data - Males

Post-dose

Group	Dose (µg/kg)		NA mmol/L	K mmol/L	CL mmol/L	CA mg/dL	PO4 mg/dL	ALP IU/L	ALT IU/L	AST IU/L	GGT IU/L	LDH IU/L
1 (VCTL)	0	MEAN	146	4.7	109	11.1	7.1	113	26	34	5	163
		SD	1.2	0.20	1.2	0.31	0.76	14.2	3.1	3.6	1.0	26.5
		N	3	3	3	3	3	3	3	3	3	3
2	10	MEAN	143	4.7	104	14.9*	5.5*	49	34	28	5	221
		SD	1.7	0.50	2.6	0.10	0.40	16.3	10.0	4.0	1.2	74.5
		N	3	3	3	3	3	3	3	3	3	3
3	30	MEAN	147	4.7	108	17.0*	5.4*	61	22	40	6	400
		SD	8.5	0.42	3.5	2.33	0.50	26.9	7.8	2.8	0.7	156.3
		N	2	2	2	2	2	2	2	2	2	2
4	90/45[a]	MEAN	147	4.2	112	16.4*	5.3*	74	46	51	6	307
		SD	4.1	0.29	3.3	1.54	0.67	42.2	29.3	21.5	1.5	152.7
		N	4	4	4	4	4	4	4	4	4	4
1 (VCTL)	0	MEAN	143	4.7	109	11.1	6.8	104	30	36	4	144
		SD	0.6	0.40	0.6	0.23	0	9.7	5.1	6.7	2.3	14.4
		N	3	3	3	3	3	3	3	3	3	3
5	5	MEAN	143	4.8	105*	12.8	5.7	87	31	39	4	131
		SD	0.7	0.57	0.7	1.20	1.41	12.7	4.2	1.4	0.7	42.4
		N	2	2	2	2	2	2	2	2	2	2

[a] dose decreased from 90 to 45 µg/kg on Day 9
* significantly different from vehicle control, $p \leq 0.05$

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 13 (cont.)

Summary of Mean Clinical Chemistry Data - Males

Post-dose

Group	Dose (µg/kg)		TBIL mg/dL	BUN mg/dL	CREA mg/dL	GLU mg/dL	TP g/dL	ALB g/dL	GLOB g/dL	A/G -	CHOL mg/dL	TG mg/dL
1 (VCTL)	0	MEAN	0.47	12	0.7	87	5.3	3.1	2.1	1.5	131	23
		SD	0.176	0.6	0.06	4.5	0.21	0.06	0.15	0.10	13.5	7.1
		N	3	3	3	3	3	3	3	3	3	2
2	10	MEAN	0.39	35	1.0	82	5.5	3.1	2.3	1.4	179	35
		SD	0.123	6.1	0.12	0.6	0.23	0.23	0.23	0.15	40.0	10.8
		N	3	3	3	3	3	3	3	3	3	3
3	30	MEAN	0.29	39	1.0	96	5.8	3.2	2.7	1.3	212	61*
		SD	0.092	1.4	0.50	20.5	0.71	0.07	0.78	0.35	31.1	21.9
		N	2	2	2	2	2	2	2	2	2	2
4	90/45[a]	MEAN	0.42	51	0.6	75	4.9	2.7	2.2	1.3	161	27
		SD	0.159	21.0	0.16	48.0	0.50	0.32	0.27	0.15	16.9	9.0
		N	4	4	4	4	4	4	4	4	4	4
1 (VCTL)	0	MEAN	0.45	15	0.8	97	5.5	3.2	2.3	1.4	143	26
		SD	0.155	1.5	0.06	7.0	0.15	0.10	0.12	0.10	16.0	8.9
		N	3	3	3	3	3	3	3	3	3	3
5	5	MEAN	0.41	16	0.8	105	5.7	3.2	2.5	1.3	158	35
		SD	0.007	0.0	0.00	9.9	0.35	0.21	0.14	0.00	9.9	5.7
		N	2	2	2	2	2	2	2	2	2	2

[a] dose decreased from 90 to 45 µg/kg on Day 9
* significantly different from vehicle control, $p \leq 0.05$

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 13 (cont.)

Summary of Mean Clinical Chemistry Data - Females

Post-dose

Group	Dose (µg/kg)		NA mmol/L	K mmol/L	CL mmol/L	CA mg/dL	PO4 mg/dL	ALP IU/L	ALT IU/L	AST IU/L	GGT IU/L	LDH IU/L
1 (VCTL)	0	MEAN	144	4.7	110	11.1	6.7	111	33	32	4	165
		SD	1.0	0.15	0.0	0.21	0.47	10.4	4.5	3.2	1.2	29.7
		N	3	3	3	3	3	3	3	3	3	3
2	10	MEAN	144	4.4	108	14.7*	5.4*	66*	28	26	5	237
		SD	0.6	0.15	1.7	0.81	0.44	7.6	7.6	4.0	1.0	156.1
		N	3	3	3	3	3	3	3	3	3	3
3	30	MEAN	144	4.7	106	15.3*	5.4*	46*	29	29	6	448*
		SD	0.6	0.10	2.3	1.33	0.06	17.6	5.0	7.2	2.0	141.8
		N	3	3	3	3	3	3	3	3	3	3
4	90/45[a]	MEAN	143	4.3	106	17.5*	4.8*	70*	34	32	5	164
		SD	3.5	0.49	4.6	0.42	0.10	15.9	0.6	9.0	1.2	106.3
		N	3	3	3	3	3	3	3	3	3	3
1 (VCTL)	0	MEAN	143	4.9	110	11.0	6.8	112	37	39	4	182
		SD	0.0	0.21	2.5	0.40	0.23	16.3	3.6	6.7	1.2	65.9
		N	3	3	3	3	3	3	3	3	3	3
5	5	MEAN	144	4.9	107	12.5	5.8*	79	43	45	4	133
		SD	2.1	0.71	2.8	0.71	0.28	6.4	4.2	11.3	1.4	9.2
		N	2	2	2	2	2	2	2	2	2	2

[a] dose decreased from 90 to 45 µg/kg on Day 8
* significantly different from vehicle control, $p \leq 0.05$

Table 13 (cont.)

Summary of Mean Clinical Chemistry Data - Females

Post-dose

Group	Dose (µg/kg)		TBIL mg/dL	BUN mg/dL	CREA mg/dL	GLU mg/dL	TP g/dL	ALB g/dL	GLOB g/dL	A/G -	CHOL mg/dL	TG mg/dL
1 (VCTL)	0	MEAN	0.37	12	0.8	92	5.3	3.3	2.0	1.6	140	17
		SD	0.035	2.5	0.10	6.7	0.23	0.20	0.12	0.12	52.4	1.5
		N	3	3	3	3	3	3	3	3	3	3
2	10	MEAN	0.48	22	0.8	91	5.6	3.4	2.2	1.5	174	24
		SD	0.090	2.6	0.06	7.6	0.15	0.12	0.10	0.12	16.4	1.7
		N	3	3	3	3	3	3	3	3	3	3
3	30	MEAN	0.52	35*	0.7	97	5.1	3.1	2.0	1.5	184	42
		SD	0.020	9.6	0.17	10.6	0.61	0.25	0.42	0.25	46.6	14.5
		N	3	3	3	3	3	3	3	3	3	3
4	90/45[a]	MEAN	0.46	31*	0.9	93	5.3	3.0	2.3	1.3	260	53*
		SD	0.081	5.5	0.21	1.5	0.15	0.06	0.10	0.06	52.0	20.7
		N	3	3	3	3	3	3	3	3	3	3
1 (VCTL)	0	MEAN	0.43	16	0.9	98	5.4	3.3	2.2	1.5	140	28
		SD	0.044	2.3	0.20	4.6	0.15	0.25	0.12	0.21	50.9	3.1
		N	3	3	3	3	3	3	3	3	3	3
5	5	MEAN	0.50	18	0.9	108	5.5	3.3	2.2	1.6	141	33
		SD	0.049	0.7	0.07	0.0	0.35	0.28	0.07	0.07	21.9	1.4
		N	2	2	2	2	2	2	2	2	2	2

[a] dose decreased from 90 to 45 µg/kg on Day 8
* significantly different from vehicle control, $p \leq 0.05$

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 14

Summary of Mean Absolute Organ Weights (g) - Males

Group	Dose (μg/kg)		Adrenals	Brain	Heart	Kidney (Left)	Kidney (Right)	Liver	Spleen	Testes	Thymus	Thyroid[a]
1 (VCTL)	0	MEAN	0.823	70.96	73.59	20.45	20.32	264.19	21.02	7.93	17.50	1.156
		SD	0.077	2.441	1.984	0.534	0.494	14.046	1.646	1.914	3.529	0.072
		N	3	3	3	3	3	3	3	3	3	3
5	5	MEAN	0.947	75.075	78.58	21.68	21.55	259.85	26.68	6.37	13.38	1.597
		SD	0.009	0.375	9.390	2.920	2.546	17.543	2.878	4.080	2.751	0.300
		N	2	2	2	2	2	2	2	2	2	2
2	10	MEAN	0.912	72.03	45.34*	20.70	19.63	192.95*	15.67	2.34	2.85*	0.839
		SD	0.127	4.356	3.066	4.553	4.214	8.836	3.125	1.468	0.554	0.085
		N	3	3	3	3	3	3	3	3	3	3
3	30	MEAN	0.959	69.18	45.75*	16.45	15.46	149.52*	14.57	1.72	3.12*	1.195
		SD	NA[b]	NA	NA	NA	NA	NA	NA	NA	NA	NA
		N	1	1	1	1	1	1	1	1	1	1
4	90/45[c]	MEAN	0.929	68.96	42.80*	18.98	18.03	157.37*	9.95*	2.22	2.06*	0.853
		SD	0.102	5.940	1.796	5.197	4.568	8.683	0.064	0.566	0.021	0.146
		N	2	2	2	2	2	2	2	2	2	2

[a] thyroids, including parathyroids
[b] NA = not applicable
[c] dose decreased from 90 to 45 μg/kg on Day 9
* significantly different from vehicle control, $p \leq 0.05$

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 14 (cont.)

Summary of Mean Absolute Organ Weights (g) - Females

Group	Dose (μg/kg)		Adrenals	Brain	Heart	Kidney (Left)	Kidney (Right)	Liver	Ovaries	Spleen	Thymus	Thyroid[a]
1 (VCTL)	0	MEAN	0.809	71.35	66.57	17.43	18.17	245.45	1.563	18.89	18.44	1.010
		SD	0.042	2.321	2.864	1.388	1.829	12.150	0.204	3.648	9.226	0.098
		N	3	3	3	3	3	3	3	3	3	3
5	5	MEAN	0.823	69.98	60.34	19.20	18.28	227.81	1.123*	16.01	10.85	1.016
		SD	0.137	NA[b]	6.223	1.655	1.612	3.111	0.047	1.442	3.790	0.138
		N	2	1	2	2	2	2	2	2	2	2
2	10	MEAN	0.747	64.08	43.69*	16.77	16.04	142.50*	0.800*	17.15	3.44*	0.957
		SD	0.087	2.996	6.062	1.822	2.244	1.656	0.107	2.856	1.130	0.245
		N	3	3	3	3	3	3	3	3	3	3
3	30	MEAN	0.871	66.80	38.74*	13.18	14.06	111.46*	0.712*	8.16*	2.05*	0.695
		SD	0.004	3.932	6.901	2.934	2.199	15.061	0.047	0.014	0.148	0.062
		N	2	2	2	2	2	2	2	2	2	2
4	90/45[c]	MEAN	0.765	69.95	39.17*	16.51	17.90	152.67*	0.732*	10.89*	1.76*	0.918
		SD	0.057	7.548	3.273	2.441	1.712	27.587	0.110	3.251	0.615	0.221
		N	3	3	3	3	3	3	3	3	3	3

[a] thyroids, including parathyroids
[b] NA = not applicable; brain of one animal inadvertently not weighed at necropsy
[c] dose decreased from 90 to 45 μg/kg on Day 8
* significantly different from vehicle control, $p \leq 0.05$

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 15

Summary of Mean Organ-to-Body Weight Ratios[a] - Males

Group	Dose (µg/kg)		FBW[b]	Adrenals	Brain	Heart	Kidney (Left)	Kidney (Right)	Liver	Spleen	Testes	Thymus	Thyroid[c]
1 (VCTL)	0	MEAN	8.61	0.010	0.83	0.86	0.24	0.24	3.07	0.24	0.09	0.20	0.013
		SD	0.090	0.001	0.036	0.014	0.006	0.006	0.181	0.022	0.022	0.042	0.001
		N	3	3	3	3	3	3	3	3	3	3	3
5	5	MEAN	8.58	0.011	0.88	0.91	0.25	0.25	3.04	0.31	0.07	0.16	0.019
		SD	0.566	0.001	0.053	0.049	0.017	0.013	0.405	0.054	0.043	0.022	0.002
		N	2	2	2	2	2	2	2	2	2	2	2
2	10	MEAN	5.03*	0.018*	1.45*	0.91	0.42	0.40	3.89	0.32	0.05	0.06*	0.017
		SD	0.677	0.004	0.237	0.115	0.136	0.126	0.661	0.102	0.031	0.013	0.002
		N	3	3	3	3	3	3	3	3	3	3	3
3	30	MEAN	4.96*	0.019*	1.40*	0.92	0.33	0.31	3.02	0.29	0.04	0.06*	0.024
		SD	NA[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		N	1	1	1	1	1	1	1	1	1	1	1
4	90/45[e]	MEAN	4.44*	0.021*	1.55*	0.97	0.42	0.40	3.57	0.23	0.05	0.05*	0.019
		SD	0.424	0.000	0.015	0.052	0.077	0.064	0.537	0.023	0.008	0.004	0.005
		N	2	2	2	2	2	2	2	2	2	2	2

[a] Organ-to-Body Weight Ratio = [Absolute Organ Weight (g) ÷ Final Body Weight (kg)] x 100
[b] FBW = Final Body Weight (kg)
[c] thyroids, including parathyroids
[d] NA = not applicable
[e] dose decreased from 90 to 45 µg/kg on Day 9
* significantly different from vehicle control, $p \leq 0.05$

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Table 15 (cont.)

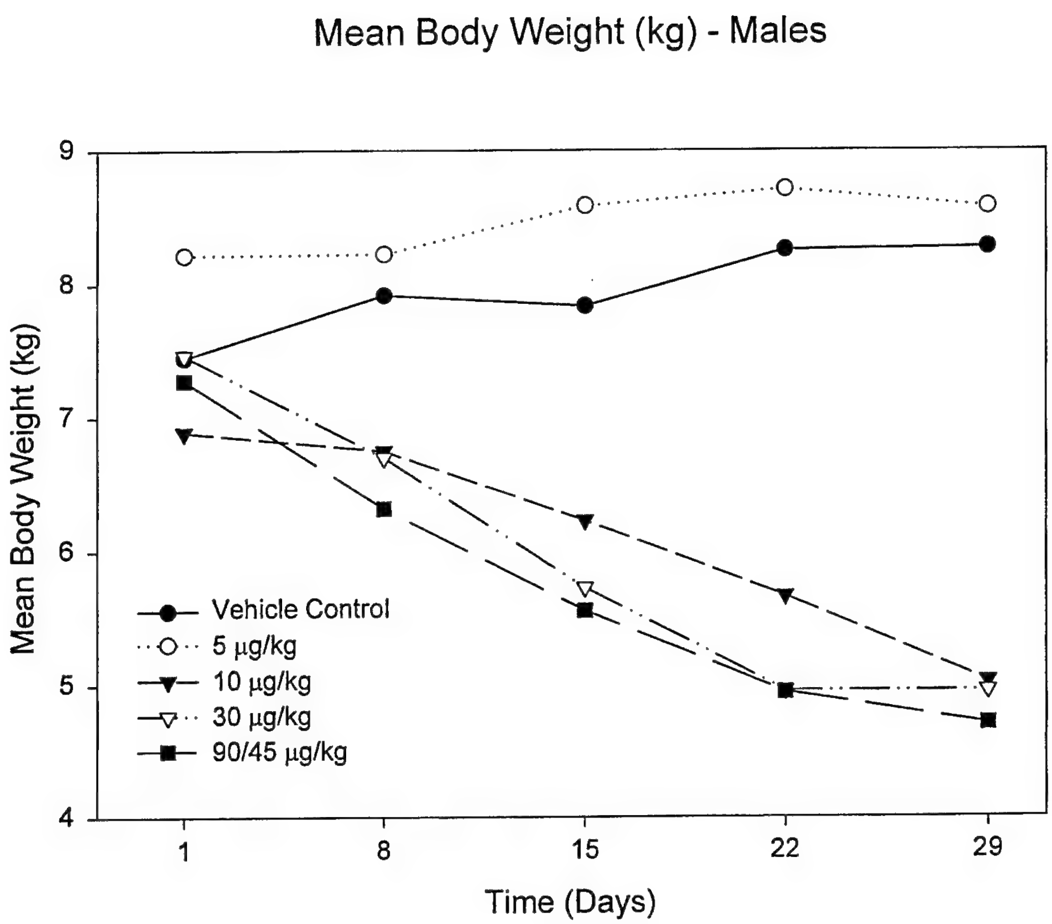
Summary of Mean Organ-to-Body Weight Ratios[a] - Females

Group	Dose (µg/kg)		FBW[b]	Adrenals	Brain	Heart	Kidney (Left)	Kidney (Right)	Liver	Ovaries	Spleen	Thymus	Thyroid[c]
1 (VCTL)	0	MEAN	7.96	0.010	0.90	0.84	0.22	0.23	3.09	0.020	0.24	0.23	0.013
		SD	0.771	0.002	0.085	0.044	0.035	0.046	0.192	0.001	0.032	0.093	0.001
		N	3	3	3	3	3	3	3	3	3	3	3
5	5	MEAN	6.97	0.012	0.96	0.87	0.28	0.27	3.28	0.016	0.23	0.15	0.015
		SD	0.410	0.003	NA[d]	0.035	0.035	0.035	0.233	0.001	0.028	0.049	0.003
		N	2	2	1	2	2	2	2	2	2	2	2
2	10	MEAN	4.91*	0.015*	1.31*	0.89	0.34*	0.33	2.93	0.016	0.35*	0.07*	0.019
		SD	0.539	0.001	0.085	0.036	0.012	0.032	0.304	0.001	0.044	0.017	0.003
		N	3	3	3	3	3	3	3	3	3	3	3
3	30	MEAN	3.84*	0.023*	1.74*	1.01	0.34	0.37*	2.90	0.019	0.21	0.06*	0.018
		SD	0.283	0.001	0.028	0.106	0.057	0.035	0.177	0.001	0.014	0.007	0.003
		N	2	2	2	2	2	2	2	2	2	2	2
4	90/45[e]	MEAN	4.20*	0.018*	1.66*	0.93	0.40*	0.43*	3.62	0.017	0.26	0.04*	0.022*
		SD	0.330	0.002	0.055	0.040	0.072	0.052	0.359	0.003	0.055	0.017	0.003
		N	3	3	3	3	3	3	3	3	3	3	3

[a] Organ-to-Body Weight Ratio = [Absolute Organ Weight (g) ÷ Final Body Weight (kg)] x 100
[b] FBW = Final Body Weight (kg)
[c] thyroids, including parathyroids
[d] NA = not applicable; brain of one animal inadvertently not weighed at necropsy
[e] dose decreased from 90 to 45 µg/kg on Day 8
* significantly different from vehicle control, $p \leq 0.05$

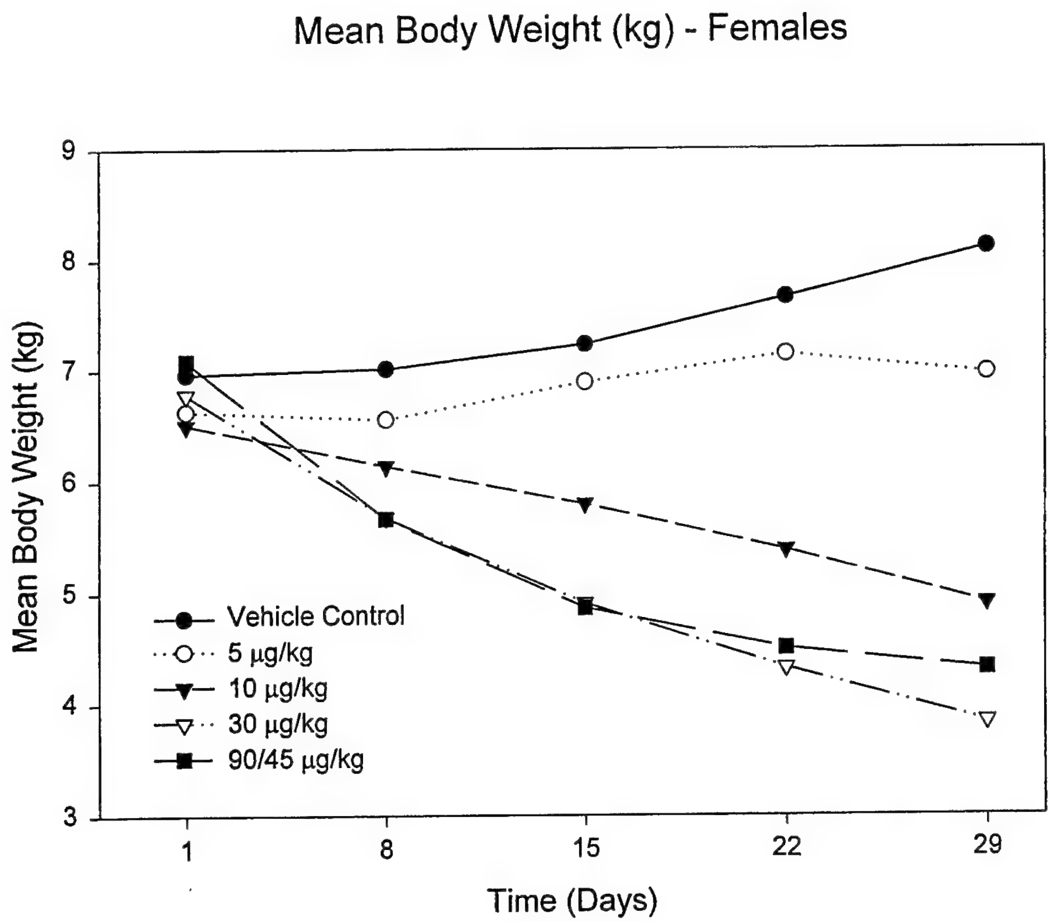
VII. FIGURES

Figure 1


Mean Body Weight (kg) - Males
Mean Body Weight (kg)
Time (Days)
9
8
7
6
5
4
1
8
15
22
29
Vehicle Control
5 µg/kg
10 µg/kg
30 µg/kg
90/45 µg/kg

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Figure 2



VIII. APPENDICES

Appendix A. Protocol, Protocol Amendments and Protocol Deviations

PROTOCOL

1. **Title:** Four-Week Oral (Gavage) Toxicity Study of 1α-Hydroxyvitamin D_5 in Beagle Dogs

2. **Sponsor:** University of Illinois at Chicago
Department of Surgical Oncology
840 South Wood Street
Chicago, Illinois 60612-7322
Attn: Tapas K. Das Gupta, M.D., Ph.D., D.Sc.

3. **Testing Facility:**

IIT Research Institute (IITRI)	Michael Reese Hospital (MRH)
10 West 35th Street	2929 South Ellis Avenue
Chicago, IL 60616	Chicago, IL 60616

4. **Objective:** To evaluate the toxicity of 1α-Hydroxyvitamin D_5 when administered orally to beagle dogs for four weeks, and to determine the reversibility of any observed toxic effects

5. **Duration:** Six Weeks

6. **Proposed Study Dates:**

   a. Animal Receipt: August 23, 2000
   b. First Day of Dosing: September 5, 2000
   c. Completion of In-Life Study: October 17, 2000
   d. Draft Report Submission: December 29, 2000

7. **Protocol Approval:**

   a. Study Director: William D. Johnson Date: 9-5-00
   William D. Johnson, Ph.D., D.A.B.T.

   b. Director, Life Sciences: [signature] Date: 9-5-00
   David L. McCormick, Ph.D., D.A.B.T.

   c. Sponsor: Tapas K. Das Gupta Date: 9/7/00
   Tapas K. Das Gupta, M.D., Ph.D., D.Sc.

8. This protocol complies with the specific requirements of the Sponsor.

9. **Test Article:**

a. Identification: The test article is identified by the Sponsor as 1α-hydroxyvitamin D_5 (1αD_5; lot 1AVD5-00A001). The test article will be supplied by the Sponsor, and will be used without further purification. The purity of 1αD_5 will be documented in a Certificate of Analysis to be provided by the Sponsor.

b. Hazards to Personnel: Routine safety procedures for handling pharmaceutical agents will be followed to insure the health and safety of personnel handling the test article.

c. Assay: The identity, purity, and stability of bulk 1αD_5 are the responsibility of the Sponsor. The homogeneity and stability of dosing formulations containing 1αD_5 will be determined. The concentration of all dosing formulations prepared for use during the study will be analyzed to verify the concentration of 1αD_5.

d. Storage: Bulk 1αD_5 will be protected from light and stored under nitrogen at approximately -60°C to -80°C). Prior to use, dosing formulations containing 1αD_5 will be stored in the dark at approximately 2°C to 5°C.

e. Disposition and Retention: All quantities of the test article which are dispensed will be documented. A sample of the corn oil vehicle used in the study will be archived at IITRI. Archiving of a retention sample of bulk 1αD_5 is the responsibility of the Sponsor.

f. Dosing Preparation: Dosing formulations of 1αD_5 will be prepared in corn oil, and will be administered using a dosing volume of 1 ml/kg. Vehicle controls will receive gavage administration of corn oil only (1 ml/kg/day).

g. Basis for Selection of Doses of Test Articles: Dose levels of 1αD_5 were selected on the basis of a 28-day oral toxicity study in rats.

h. Route: The oral route is the intended clinical route of administration for 1αD_5.

i. Test Article Return: Upon completion of the study, remaining test article will be returned to the Sponsor.

10. **Test System:**

a. Test Animals: Sixteen male and 16 female purebred beagle dogs (Ridglan Farms, Inc., Mt. Horeb, WI), 5 to 6 months old at arrival, will be used in this study. All animals are immunized by the supplier against distemper, leptospirosis, adenovirus, coronovirus, parainfluenza, rabies, and parvovirus. Dogs will weigh approximately 7 to 9 kg at the initiation of dosing.

b. Justification of Species Selected: The dog is a standard non-rodent model system used for toxicity studies, and is accepted by the United States Food and Drug Administration (FDA) as a non-rodent species for preclinical safety assessments.

c. Justification of Number of Animals: The number of animals used is the minimum necessary to satisfy scientific principles and regulatory requirements. To the knowledge of the Sponsor and the Study Director, conduct of this study will result in no unnecessary duplication of existing data with regard to species, test article, dose(s), routes, and duration of administration.

d. Housing: Dogs will be housed individually in stainless steel cages equipped with automatic watering systems. Excrement pans under dog cages will be cleaned daily. Dogs will be housed in accordance with standards set forth in the Guide for Care and Use of Laboratory Animals (National Research Council, 1996) and by the United States Department of Agriculture through the Animal Welfare Act (7 USC 2131-2156, 1985) and Animal Welfare Standards incorporated in Title 9, CFR, Part 3, 1991.

e. Food: Certified Canine Diet #5007. Approximately 300 g of food will be made available to each dog daily for a minimum of 2 hours. Each lot of diet is analyzed for contaminants to ensure that none is present at a concentration that would be expected to interfere with the conduct or purpose of this study. Analytical data from the lots of diet to be used in the study will be maintained in the study notebook.

f. Water: City of Chicago water will be provided *ad libitum* to all dogs by an automatic watering system. Supply water is analyzed for contaminants as defined by the U.S. EPA "National Interim Primary Drinking Water Regulations" (Title 40, CFR, Parts 141.1 (b) and 141.12). Water analysis records are retained on file at Michael Reese Hospital. No contaminants expected to interfere with the study are known to be present in the water.

g. Animal Identification: Each dog will be identified by USDA tattoo number and/or letter in the left or right ear. Each dog will also be assigned a unique number within the study. All cages will be identified by IITRI Project Number, Study Number, Group, Animal Number, and Sex.

h. Environmental Control: Temperature and relative humidity in the animal room will be recorded manually each day. A 12-hour light/dark cycle (maintained with an automatic timer) will be used. Animal rooms will be held within a temperature range of approximately 18°C to 26°C, and a humidity range of approximately 30 to 70%.

11. **Experimental Design:** The study design can be summarized as follows:

Group	1αD$_5$ Dose (μg/kg body weight)	No. of Animals Main Study (M + F)	No. of Animals Recovery (M + F)
1	0 (Control)	3 + 3	2 + 2
2	10	3 + 3	--
3	30	3 + 3	--
4	90	3 + 3	2 + 2

12. Methods:

a. Quarantine: Animals purchased for this study will be held in quarantine for approximately two weeks prior to administration of test article. During the quarantine period, animals will be observed at least once daily for mortality or evidence of moribundity. At the end of the quarantine period, dogs will be randomly assorted into groups using a computerized randomization procedure that blocks for body weights. Prior to randomization, each dog will receive a detailed physical examination to ensure its suitability as a test animal.

b. Administration: $1\alpha D_5$ will be administered daily by gavage (in a vehicle of corn oil [1 ml/kg body weight]) for a minimum of 28 consecutive days; vehicle control dogs will receive 1 ml corn oil per kg body weight. At the end of the exposure period, recovery animals in groups 1 (control) and 4 (high dose) will be held for two weeks without further dosing.

c. Moribundity/Mortality Observations: During the quarantine period, all animals will be observed at least once daily for mortality or evidence of moribundity. Throughout the treatment and recovery periods, all animals will be observed twice daily for mortality or evidence of moribundity. Any abnormal clinical signs will be recorded. Twice daily mortality/ moribundity checks will be separated by a minimum of four hours.

d. Moribund Animals: During the moribundity/mortality observations, any animal judged not likely to survive until the next scheduled observation period will, upon consent of the Study Director or his designate (Study Veterinarian or Study Pathologist), be removed from the study, euthanized, and necropsied. These animals will be recorded in the study notebook as being euthanized *in extremis*. Dead animals will be removed immediately for necropsy and the death will be recorded in the study notebook.

e. Injured or Diseased Animals: Animals on test will be treated for disease or injury within the standards of accepted veterinary practice. Approval of the Study Sponsor will be obtained prior to initiation of any treatment that could impact the results of the toxicity bioassay. A complete record of the circumstances, treatment, and disposition of any affected animals will be made in the study notebook. Any dogs which pose a potential infectious threat to other study animals will be isolated.

f. Clinical Observations: Cageside clinical observations will be performed daily during the treatment and recovery periods. A detailed clinical and physical examination will be performed on all animals once during the quarantine period (pretest) and weekly throughout the treatment and recovery periods.

g. Body Weight Measurements: Animals will be weighed once during quarantine (pretest), weekly during the treatment and recovery periods, and prior to the scheduled Main Study and Recovery necropsies.

h. Food Consumption Measurements: Food consumption will be measured daily and reported weekly for each animal during the treatment and recovery periods.

i. Ophthalmic Examinations: Indirect funduscopic examinations will be performed on all dogs during quarantine (pretest) and on all surviving dogs during the final week of the treatment period. If test article related ophthalmic effects are seen during the final week of the treatment period, examinations will also be performed during the final week of the recovery period.

j. Electrocardiographic evaluations: Electrocardiographic evaluations will be performed on all dogs during quarantine (pretest) and on all surviving dogs during the last week of treatment. If test article related electrocardiographic effects are seen during the treatment period, evaluations will also be performed during the final week of the recovery period. Analysis will include heart rate and rhythm, amplitude of the P wave and QRS complex, and duration of the P wave, PR, QRS, and QT intervals.

k. Clinical Pathology: Urine samples for urinalysis and blood samples for clinical chemistry, hematology, and coagulation parameter evaluations will be obtained from all dogs during the quarantine period, all surviving Main Study and Recovery dogs prior to the terminal necropsy of the Main Study animals, and on all surviving recovery animals during the final week of the recovery period. Dogs will be fasted prior to blood collection. Blood samples for clinical chemistry, hematology, and coagulation parameters will be collected via the jugular or cephalic vein. The following clinical pathology tests will be performed:

1. Clinical Chemistry:

Calcium	Alanine aminotransferase	Albumin (A)
Inorganic phosphorus	Aspartate aminotransferase	Globulin (G)
Chloride	Gamma-glutamyl transpeptidase	A/G ratio
Sodium	Lactate dehydrogenase	Creatinine
Potassium	Cholesterol	Total bilirubin
Glucose	Triglycerides	Total protein
Alkaline phosphatase		Urea nitrogen

2. Hematology:

Erythrocyte count	Mean corpuscular volume
Erythrocyte morphology	Mean corpuscular hemoglobin
Absolute white blood cell count	Mean corpuscular hemoglobin concentration
Relative white blood cell count	Platelet count
Hematocrit	Reticulocyte count
Hemoglobin	

3. Coagulation:

Prothrombin time	Fibrinogen	Activated partial thromboplastin time

4. Urinalysis:

Volume	Protein	Leukocytes
Appearance	Glucose	Occult blood
Color	Bilirubin	Microscopic examination of sediment
Refractive index	Urobilinogen	
Specific gravity	Nitrite	
pH	Ketones	

13. **Postmortem**:

a. Necropsy: All dogs, including those found dead or euthanized moribund, will receive a complete necropsy. Terminal necropsies will be performed on all surviving Main Study dogs on day 29, and on all surviving Recovery dogs on day 43. Necropsy will include examination of the external surface of the body, all orifices, the cranial, thoracic, and peritoneal cavities, and their contents. Prior to scheduled necropsies, surviving dogs will be fasted overnight and euthanized by barbituate overdose. All tissues collected will be fixed in 10% neutral buffered formalin.

b. Tissues Preserved:

*Adrenals
Aorta (thoracic)
*Brain (entire)
Epididymides
Esophagus
Eyes with optic nerves
Femur, including diaphysis with marrow cavity and epiphysis (femoral condyle with epiphyseal cartilage plate, articular cartilage, and articular surface)
Gall bladder
*Heart
Intestine
- Cecum
- Colon
- Duodenum (with bile & pancreatic ducts)
- Ileum
- Jejunum
- Rectum

*Kidneys (weighed separately)
*Liver (right medial and left lateral lobes)
Lungs (left apical [infused] and left diaphragmatic [non-infused] lobes) and bronchi
Lymph nodes (bronchial, mandibular, mesenteric)
Mammary gland (left inguinal, with skin)
*Ovaries and fallopian tubes
Pancreas
**Parathyroids (weighed with thyroids)
Pituitary
Prostate
Salivary gland (mandibular)
Sciatic nerve
Skeletal muscle
Skin (dorsal thorax, elbow)
Spinal cord (cervical and thoracic)
*Spleen
Sternum (bone marrow)
Stomach (fundic and pyloric regions)
*Testes
*Thymus
**Thyroids (weighed with parathyroids)
Tongue
Tonsil (palatine)
Trachea
Ureter
Urinary bladder
Uterus (corpus and cervix)
Vagina
Gross lesions
Tissue masses and regional lymph nodes

Organs marked with an asterisk (*) will be weighed at necropsy. To prevent possible tissue damage associated with weighing, the thyroid and parathyroids (**) will be weighed after approximately 24 hours of formalin fixation. A bone marrow smear will be prepared from the rib of each dog and stained with Wright-Giemsa stain for possible evaluation.

c. Histopathologic Evaluation: The tissues listed above from all Main Study dogs in the control (group 1) and high dose (group 4) groups will be evaluated histopathologically by a board-certified veterinary pathologist. Histopathologic evaluations in dogs from the low and mid dose groups and in the Recovery groups will be limited to gross lesions and identified target tissues. Tissues to be examined histopathologically will be embedded in paraffin, processed by routine histologic methods, and stained with hematoxylin and eosin.

d. Statistical Analysis: Statistical analysis of continuous data will be performed using analysis of variance, with post-hoc comparisons made using Dunnett's test. A minimum significance level of $p < 0.05$ will be used for all comparisons.

14. **Quality Assurance:** This study will be audited by the IITRI Quality Assurance Unit to assure adherence with Good Laboratory Practice Regulations, adherence to the study protocol, and compliance with Standard Operating Procedures.

15. **Reports:** A draft version of the report will be prepared and submitted to the Sponsor for review and evaluation prior to submission of the final study report. Information in the report will include, but not be limited to, the following:

    a. Species and strain of animal used
    b. Toxic response data by sex and dose
    c. Date of death during the study or whether animals survived to termination
    d. The period of observation of each abnormal sign and its subsequent course
    e. Food consumption and body weight data
    f. Formulation analysis data
    g. Results of ophthalmological and electrocardiographic evaluations
    h. Hematology, clinical chemistry, and coagulation tests employed with results
    i. Necropsy findings
    j. Detailed description of results, where appropriate
    k. Statistical treatment of results, where appropriate.

    Following Sponsor review of the Draft Report, a Final Report will be submitted to the Sponsor. The Final Report will contain a statement prepared and signed by the IITRI Quality Assurance Unit, and the signatures of the Study Director and Director of Life Sciences.

16. **Alteration of Design:** Alterations in the protocol may be made as the study progresses. No changes in the protocol will be made without the specific written consent of the Sponsor.

17. **Data Notebooks:** All original data will be maintained in loose-leaf notebooks. These will include, but not necessarily be limited to, the following:

    a. The original signed protocol and any amendments and deviations.
    b. Animal receipt records.
    c. Animal care records.
    d. Test article preparation and administration data.
    e. Analytical chemistry data.
    f. Daily moribundity/mortality data.

g. Clinical observation data.
h. Body weight data.
i. Food consumption data
j. Ophthalmology data.
k. Electrocardiography data
l. Clinical pathology data.
m. Necropsy and histopathology data.

18. **Data Retention:** All raw data generated at IITRI or MRH, specimens, and a copy of the final report from the study will be archived in the IITRI archives (10 West 35th Street, Chicago, IL) for a period of 5 years from the date of completion of the study. At that time, the Sponsor will be contacted in order to determine the final disposition of the archival materials. The Sponsor will be responsible for all costs associated with continued storage of the archival materials in the IITRI archives or for the shipment of these materials to another storage facility. The IITRI Quality Assurance Unit will maintain a complete record of the disposition of all archival materials.

19. **Personnel:** *Curricula vitae* for all personnel involved in the execution of the study are on file at IITRI or MRH.

20. **Compliance Statement:** This study will be conducted in compliance with the U.S. FDA Good Laboratory Practice Regulations set forth in Part 58 of Title 21 of the Code of Federal Regulations.

PROTOCOL AMENDMENT

IITRI Project No.: 1209

Study Number: 2

Protocol Amendment No.: 1

Study Title: Four-Week Oral (Gavage) Toxicity Study of 1α-Hydroxyvitamin D_5 in Beagle Dogs

The following changes are being made to the protocol:

11. Experimental Design: Because of toxicity (i.e., mortality of two female dogs and body weight loss of both male and female dogs) at the high dose (90 μg/kg) level during the first week of the study, the high dose recovery group will be eliminated, and the high dose level for all surviving high dose dogs will be decreased to 45 μg/kg body weight for the remainder of the 28-day dosing period, beginning September 13, 2000 (study day 9 and 8 for males and females, respectively). In addition, the two dogs per sex in the vehicle control group originally designated as recovery animals will be dosed with the test article at a level of 5 μg/kg for 28 days. Thus, the study design is being modified as follows:

Group	$1\alpha D_5$ Dose (μg/kg body weight)	No. of Animals (M & F)
1	0 (Control)	3 + 3
2	10	3 + 3
3	30	3 + 3
4	45	5 + 3
5	5	2 + 2

4. **Objective**: To evaluate the toxicity of 1α-Hydroxyvitamin D_5 when administered orally to beagle dogs for four weeks

5. **Duration**: Four Weeks

6. **Proposed Study Dates**:

   c. Completion of In-Life Study: October 11, 2000

Reason for Change: The high dose level (90 μg/kg) is being decreased due to mortality. The control dogs originally designated as recovery animals are being dosed with test article at a level of 5 μg/kg in an attempt to obtain a no effect level. Mortality of two high dose female dogs and dosing of the recovery control dogs with test article eliminated the recovery group animals.

PROTOCOL AMENDMENT

IITRI Project No.: 1209

Study Number: 2

Protocol Amendment No.: 1

Study Title: Four-Week Oral (Gavage) Toxicity Study of 1α-Hydroxyvitamin D_5 in Beagle Dogs

9.f. Dosing Preparation; 12.b. Administration: Effective September 13, 2000 (study day 9 and 8 for males and females, respectively), dosing formulations of $1\alpha D_5$ will be administered at a dosing volume of 0.5 or 1 ml/kg body weight.

Reason for Change: Decreasing the high dose from 90 to 45 μg/kg on study day 8 (females) or 9 (males) and dosing the recovery control dogs at 5 μg/kg was facilitated by decreasing the dosing volume of the high dose formulation (90 μg/ml) and the low dose formulation (10 μg/ml) from 1 to 0.5 ml/kg body weight.

13.b. Tissues Preserved:

Lungs (right apical [infused] and right diaphragmatic [non-infused] lobes) and bronchi
*Parathyroids (weighed with thyroids)
*Thyroids (weighed with parathyroids)

Organs marked with an asterisk (*) will be weighed at necropsy. A bone marrow smear will be prepared from the rib of each dog and stained with Wright-Giemsa stain for possible evaluation.

Reason for Change: 1) Right lung lobes are separate while lobes are usually fused on the left; 2) Per SOP NS-126R1.

Because the recovery groups for the study have been eliminated, reference to recovery in other sections of the protocol are no longer applicable.

APPROVAL:

a. Study Director: William D. Johnson — 9-15-00
William D. Johnson, Ph.D., D.A.B.T. — Date

b. Director, Life Sciences: D L McCormick — 9-15-00
David L. McCormick, Ph.D., D.A.B.T. — Date

c. Sponsor: Tapas K. Das Gupta — 10/02/00
Tapas K. Das Gupta, M.D., Ph.D., D.Sc. — Date

PROTOCOL AMENDMENT

IITRI Project No.: 1209

Study Number: 2

Protocol Amendment No.: 2

Study Title: Four-Week Oral (Gavage) Toxicity Study of 1α-Hydroxyvitamin D_5 in Beagle Dogs

The following changes are being made to the protocol:

12.i. Ophthalmic Examinations: Indirect funduscopic examinations will be performed on all dogs during quarantine (pretest) and on all surviving dogs during the final week of treatment, except the exams will be done during the 3rd week of treatment for the dogs in the 5 μg/kg dose group.

Reason for Change: Dosing of the 5 μg/kg dose group dogs was initiated approximately one week later than the other dose groups.

12.k. Clinical Pathology: Blood samples for clinical chemistry, hematology, and coagulation parameter evaluations will be obtained from all surviving dogs during the final week of treatment. Urine samples for urinalysis will be collected from all surviving dogs at the time of necropsy.

Reason for Change: Collection of blood samples during the last week of treatment allows for clinical pathology evaluation of the animal prior to sacrifice. Collection of urine samples at necropsy will allow collection of a sterile sample.

APPROVAL:

a.	Study Director:	William D. Johnson William D. Johnson, Ph.D., D.A.B.T.	10-2-00 Date
b.	Director, Life Sciences:	D L McCormick David L. McCormick, Ph.D., D.A.B.T.	10/2/00 Date
c.	Sponsor:	Tapas. K. Das Gupta Tapas K. Das Gupta, M.D., Ph.D., D.Sc.	10/12/00 Date

PROTOCOL AMENDMENT

IITRI Project No.: 1209

Study Number: 2

Protocol Amendment No.: 3

Study Title: Four-Week Oral (Gavage) Toxicity Study of 1α-Hydroxyvitamin D_5 in Beagle Dogs

The following change is being made to Section 13.c. (Histopathologic Evaluation) of the protocol:

13.c. Histopathologic Evaluation: Tissues from all dogs in the control (group 1; 0 μg/kg) and low-mid (group 2; 10 μg/kg) dose groups, and from the two dogs (animal numbers 1261 male and 1239 female) in the high-mid (group 3; 30 μg/kg) dose group which were sacrificed moribund will be evaluated histopathologically by a board-certified veterinary pathologist. In addition, target tissues and gross lesions from dogs in the low dose group (group 5; 5 μg/kg) will also be evaluated histopathologically.

Reason for Change: All dogs in the high dose (group 4; 90/45 μg/kg) group either died or were sacrificed moribund prior to study termination, or were severely debilitated at the time of terminal sacrifice.

APPROVAL:

a. Study Director: William D. Johnson — 1-23-01

William D. Johnson, Ph.D., D.A.B.T. — Date

b. Director, Life Sciences: [signature] — 1/23/01

David L. McCormick, Ph.D., D.A.B.T. — Date

c. Sponsor: Tapas.K. Das Gupta — 1/26/01

Tapas K. Das Gupta, M.D., Ph.D., D.Sc. — Date

PROTOCOL DEVIATION

IITRI Project No.: 1209

Study Number: 2

Protocol Deviation No.: 1

Study Title: Four-Week Oral (Gavage) Toxicity Study of 1α-Hydroxyvitamin D_5 in Beagle Dogs

10.e. Food: On the first day of dosing of the male dogs (9-5-00), food was made available to several dogs for less than the minimum of 2 hours as specified in the protocol.

This deviation did not affect the integrity of the study.

William D. Johnson   9-15-00

William D. Johnson, Ph.D., D.A.B.T.   Date
Study Director

PROTOCOL DEVIATION

IITRI Project No.: 1209

Study Number: 2

Protocol Deviation No.: 2

Study Title: Four-Week Oral (Gavage) Toxicity Study of 1α-Hydroxyvitamin D_5 in Beagle Dogs

12.b. Administration: Animals in the high dose group (90 μg/kg) were not dosed on September 12, 2000 (study day 8 for males and 7 for females) due to toxicity at this dose level. Dosing of these animals was resumed on September 13, 2000 at a level of 45 μg/kg. Therefore, 1αD_5 will be administered to the high dose group animals daily for a minimum of 28 days, however not for 28 consecutive days as per the protocol.

This deviation did not affect the integrity of the study.

William D. Johnson — 9-15-00

William D. Johnson, Ph.D., D.A.B.T. — Date

Study Director

Appendix B. Dose Formulation Analysis Report and Certificates of Analysis

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

I Analysis of Bulk Test Article: The identity, purity and stability of the test article, 1α-hydroxyvitamin D_5 (1αD5), were the responsibility of the Sponsor. A Certificate of Analysis for lot number 1AVD5-00A001, with supporting chromatograms, as well as a Certificate of Analysis for the corn oil (vehicle) used in this study are included at the end of this Appendix.

II Analysis of 1αD5 Formulations: A stock solution of 1αD5 was prepared by dissolving approximately 20 mg of the test article (1α-hydroxyvitamin D_5; Lot no. 1AVD5-00A001; received 1/26/00) in 4 ml ethanol. The stock solution was stored at -20° C when not in use. The stock solution was brought to room temperature before use on each day of analysis to prepare the standard curve. Standard curve calibrators were prepared at approximately 1, 2, 4, 20, 50, 100 and 200 µg/ml by dilution with acetone followed by mixing with corn oil. A typical standard curve is presented in Figure B-1.

1αD5 formulations were prepared for analysis by dilution in acetone. Acetone (1 ml) was transferred to a culture tube. Test article formulation (1 ml) was added via pipettor and the pipettor tip was rinsed repeatedly with the acetone. An aliquot was then transferred to an HPLC vial for injection into the HPLC. The 1αD5 concentration was determined by comparing the peak area of unknown samples to the response from the linear regression of the standard curve.

HPLC conditions were based on those supplied by the Sponsor for the analysis of 1αD5. A gradient was added to the HPLC mobile phase in order to elute strongly retained components of the corn oil from the HPLC column.

The HPLC conditions were:

Column:	Phenomenex Sphereclone ODS-2, 5µ, 250 x 4.6 mm i.d.
Column heater temperature:	30°C
Mobile phase A:	Acetonitrile:methanol:Milli-Q water (575:335:90 v/v/v)
Mobile phase B:	Acetonitrile:methanol (650:375 v/v)
Flow rate:	1.0 ml/min
Detection:	UV absorbance at 254 nm
Injection volume:	10 µl
Run time:	132 min

On each day of sample analysis, a complete standard curve was run, along with quality control (QC) samples and dilute formulation samples. System suitability tests consisted of peak symmetry determination and five sample injections to determine system reproducibility.

III Homogeneity of 1αD5 Formulations: Homogeneity was determined on the 30 μg/ml dose formulation on the first day of preparation by taking duplicate samples from the top, middle and bottom of the container used to prepare the dose formulation. Samples were diluted and analyzed as described previously. This dose formulation was homogenous (R.S.D., 2%). The complete results are presented in Table B-1.

IV Stability of 1αD5 Formulations: After 1 week, samples from the first dose formulation were analyzed for stability. Samples were diluted and analyzed as described previously. The dose formulations were stable (99-109% of initial concentrations). The complete results are presented in Table B-2.

V Dose Formulation Analysis: Concentration of the dose formulations used in this study were determined as described in Section B. Dose formulations were diluted in 1 ml acetone and injected into the HPLC. Duplicate samples were collected from each dose formulation.

Dosing formulations used during the study were prepared weekly and analyzed. Because of the long run time (132 min/sample), it was not possible to complete analysis of dosing formulations prior to dosing. The analyzed concentration of all dosing formulations was within 10% of theoretical except for the 30 μg/ml dose prepared on 9/01/00 and analyzed for homogeneity which was 89% (reanalyzed on 9/8/00, 97%), 10 μg/ml dose prepared on 9/8/00 (112%), 5 μg/ml dose prepared 9/18/00 (89%) and 5 μg/ml dose prepared on 9/29/00 (120%). Results of individual analyses are presented in Table B-3. Typical chromatograms for the dose formulations are shown in Figure B-2.

Michael Cwik, Ph.D. Date
Senior Chemist
Life Sciences Operation

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix Table B-1

1α-Hydroxyvitamin D5 Dose Formulation Homogeneity Analysis
Performed 09/01/00

Theoretical concentration	**Replicate**	**Determined concentration (μg/ml)**
30.0 μg/ml	Top 1	27.3
	Top 2	27.5
	Middle 1	26.8
	Middle 2	26.2
	Bottom 1	27.1
	Bottom 2	26.8
	Mean	26.8
	S.D.	0.60
	R.S.D.	2%
	% of Target	89%

Appendix B - Dose Formulation Analysis Report

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix Table B-2

1α-Hydroxyvitamin D5 Dose Formulation Stability Analysis

Date of Analysis	09/01/00	09/08/00
Date of Preparation	09/01/00	09/01/00
10 μg/ml 1α-hydroxyvitamin D5 formulations		
Analyzed concentration (μg/ml)		
Replicate 1	9.2	10.1
Replicate 2	9.4	9.7
mean	9.3	9.9
% of Day 0		106
30 μg/ml 1α-hydroxyvitamin D5 formulations		
	Analyzed concentration	
Replicate 1	27.3	28.9
Replicate 2	27.5	29.5
Replicate 3	26.8	
Replicate 4	26.2	
Replicate 5	27.1	
Replicate 6	26.0	
mean	26.8	29.2
% of Day 0		109
90 μg/ml 1α-hydroxyvitamin D5 formulations		
Analyzed concentration (μg/ml)		
Replicate 1	87.1	88.6
Replicate 2	88.4	85.1
mean	87.8	86.9
% of Day 0		99

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix Table B-3

1α-Hydroxyvitamin D5 Dose Formulation Analysis

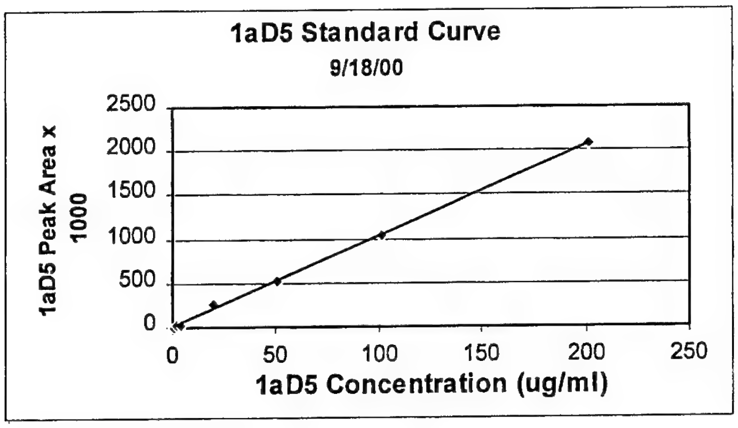
Date of Analysis	09/01/00	09/08/00	09/18/00	09/22/00	09/29/00
Date of Preparation	**09/01/00**	**09/08/00**	**09/18/00**	**09/22/00**	**09/29/00**
5 µg/ml 1α-hydroxyvitamin D5 formulations					
Analyzed concentration (µg/ml)					
Replicate 1			4.3	4.9	6.1
Replicate 2			4.6	4.6	5.9
mean			4.5	4.8	6.0
% of Target			89	95	120
10 µg/ml 1α-hydroxyvitamin D5 formulations					
Analyzed concentration (µg/ml)					
Replicate 1	9.2	11.3	10.3	9.8	10.9
Replicate 2	9.4	11.0	9.1	9.7	11.2
mean	9.3	11.2	9.7	9.8	11.1
% of Target	93	112	97	98	111
30 µg/ml 1α-hydroxyvitamin D5 formulations					
Analyzed concentration (µg/ml)					
Replicate 1	28.9[a]	32.6	32.6	30.2	31.9
Replicate 2	29.5[a]	32.2	30.6	29.6	32.2
mean	29.2	32.4	31.6	29.9	32.1
% of Target	97	108	105	100	107
45 µg/ml 1α-hydroxyvitamin D5 formulations					
Analyzed concentration (µg/ml)					
Replicate 1			45.5	44.8	47.7
Replicate 2			44.9	45.1	49.4
mean			45.2	45.0	48.6
% of Target			100	100	108
90 µg/ml 1α-hydroxyvitamin D5 formulations					
Analyzed concentration (µg/ml)					
Replicate 1	87.1	95.2			
Replicate 2	88.4	94.3			
mean	87.8	94.8			
% of Target	98	105			

[a]–Results of reanalysis performed 9/08/00

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix Figure B-1

HPLC Calibration Curve for 1αD5 Formulation

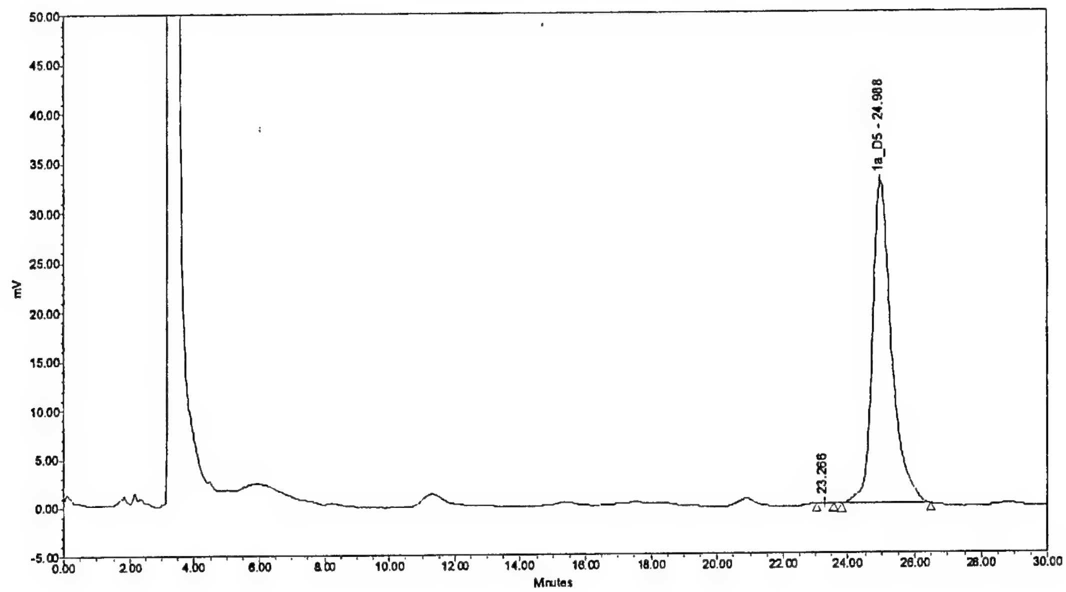


Appendix B - Dose Formulation Analysis Report

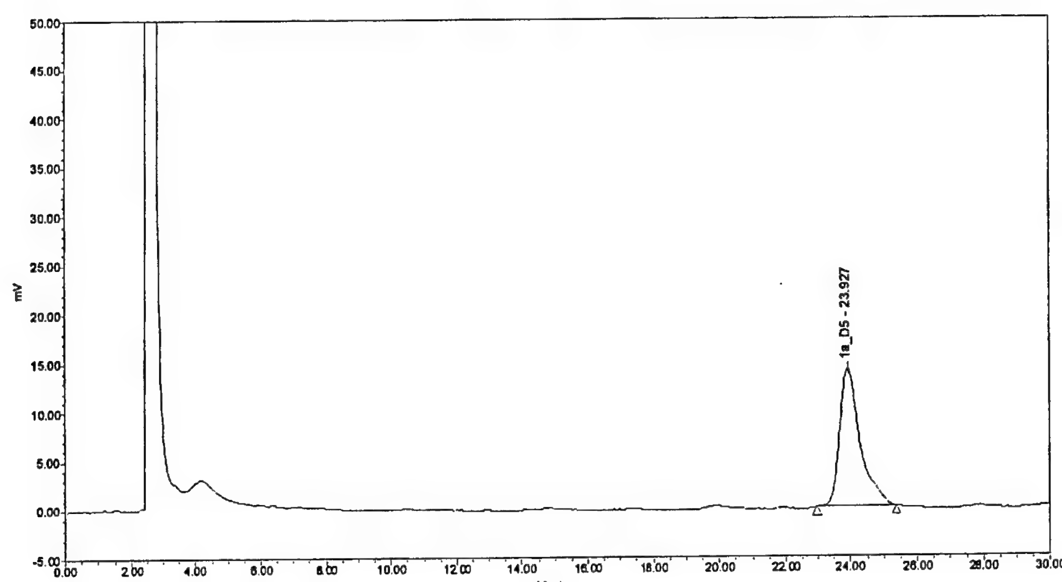
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix Figure B-2

Chromatograms for Dose Formulation Samples



45 µg/ml, 09/18/00



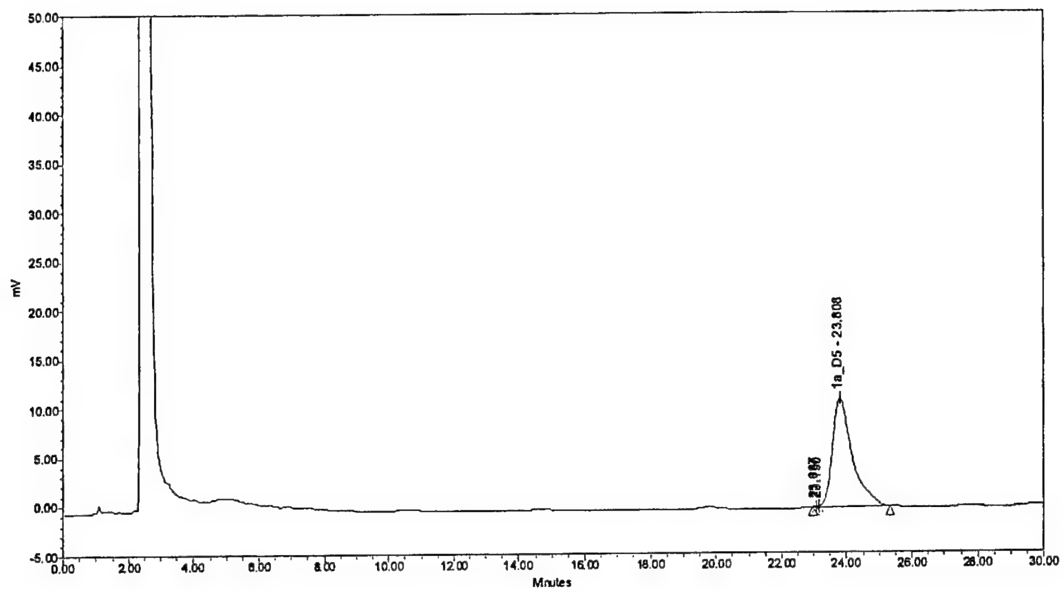
30 µg/ml, 09/18/00

Appendix B - Dose Formulation Analysis Report

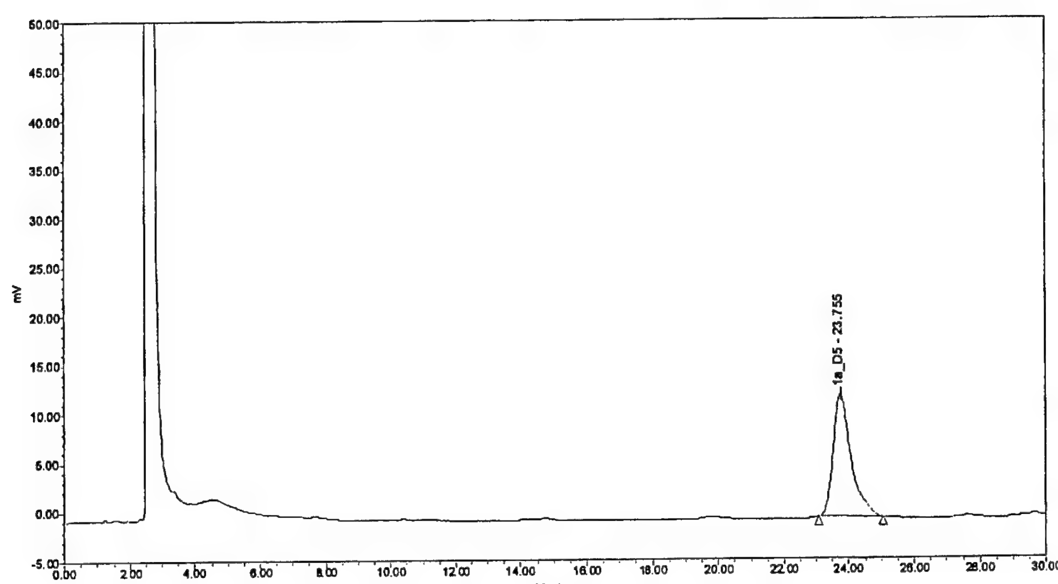
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix Figure B-2 (cont.)

Chromatograms for Dose Formulation Samples



10 μg/ml, 09/18/00



5 μg/ml, 09/18/00

SynQuest, Inc.
Enterprise Center
Illinois Medical District
2225 W. Harrison Street
Chicago, Illinois 60612

Tel: (312) 421-1819
Fax: (312) 421-8177

CERTIFICATE OF ANALYSIS

NAME:	1α-Hydroxyvitamin D_5
LOT NUMBER:	1AVD5-00A001
APPEARANCE:	White solid
PURITY BY HPLC:	96.4%
MELTING POINT:	148°C - 150°C
IR:	See attached spectrum
^{1}H NMR:	See attached spectrum

PREPARED BY: Tania Parvu DATE: 1/5/00

APPROVED BY: Jodie Lahamme DATE: 1/5/00

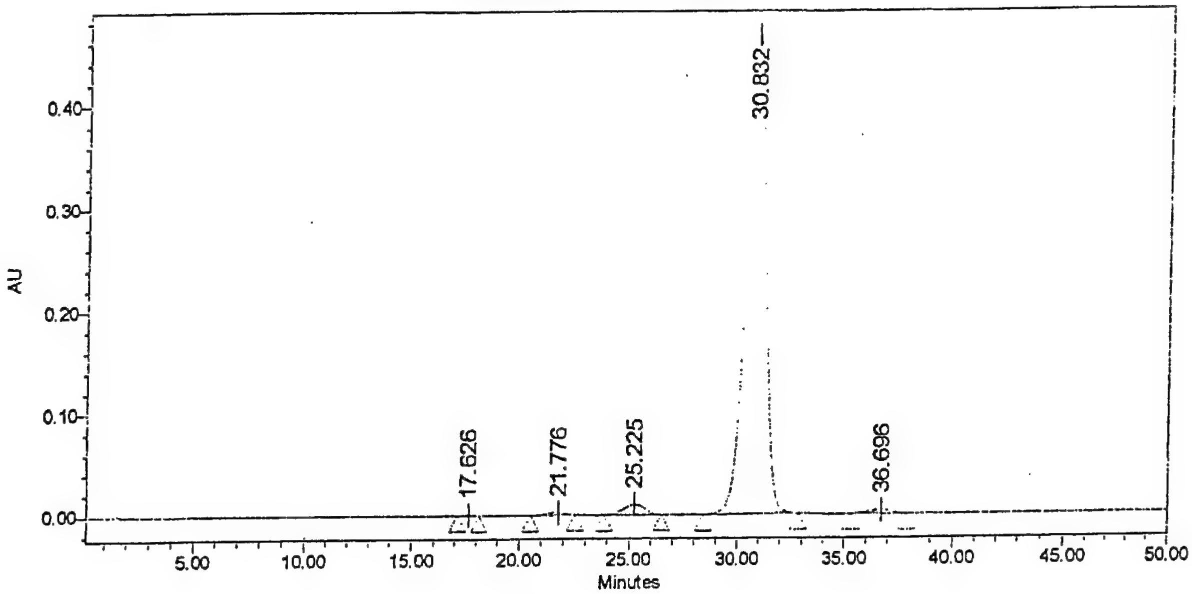
SynQuest, Inc.

Current Date 1/5/00

Sample Information

SampleName	1alpha(OH)05	Sample Type	Unknown
Vial	1	Date Acquired	1/5/00 3:31:10 PM
Injection	1	Acq Method Set	WatersVitaminD
Injection Volume	20.00 ul	Processing Method	VitaminD
Channel	996	Date Processed	1/5/00 4:32:32 PM
Run Time	50.0 Minutes		

Auto-Scaled Chromatogram



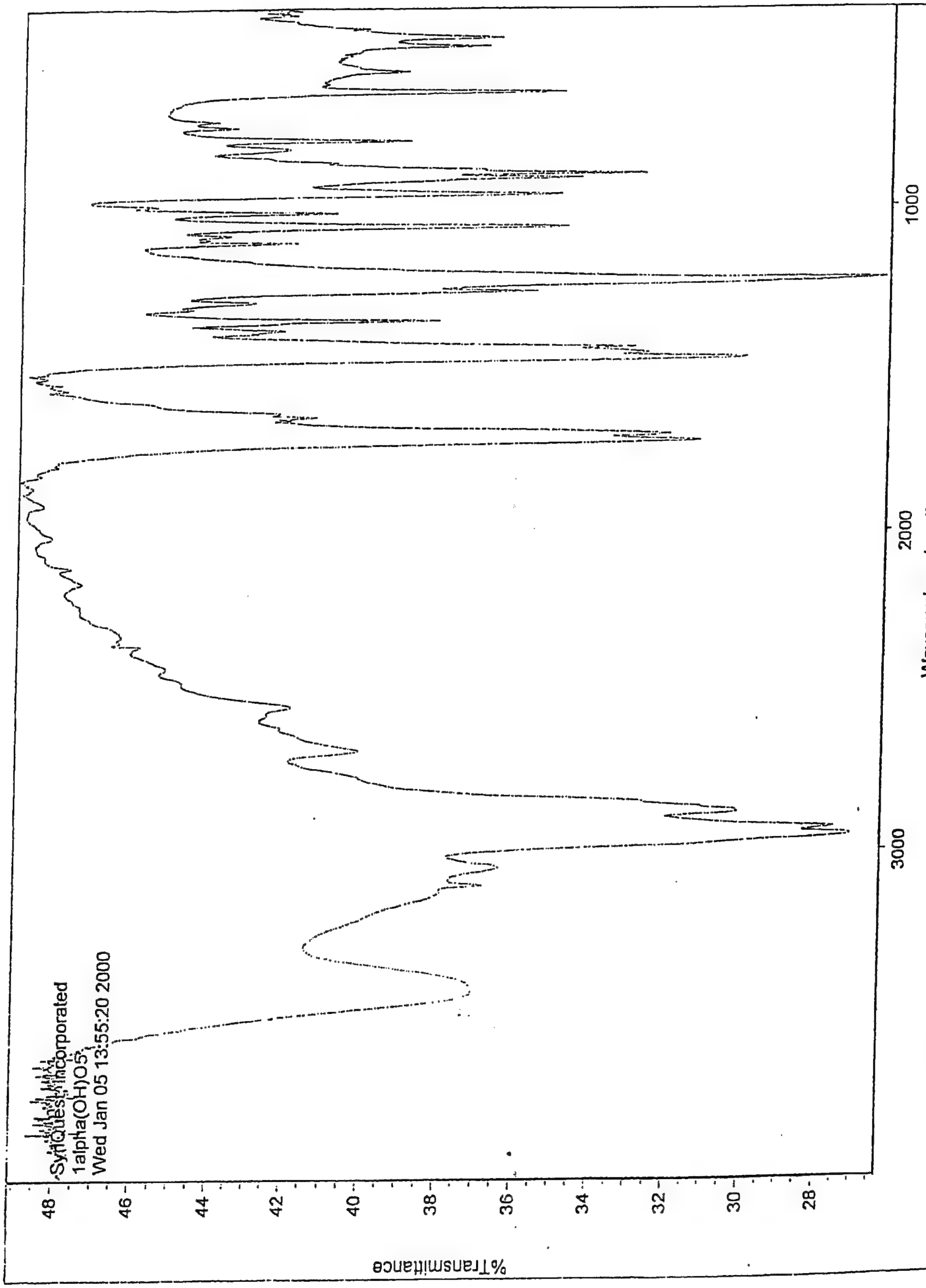
Peak Results

	Name	Retention Time	Int Type	Area	Height	% Area	Amount	Units
1		17.626	BB	19381	642	0.07		
2		21.776	BB	137978	2288	0.46		
3		25.225	BB	667804	10033	2.25		
4		30.832	BB	28644183	466747	96.38		
5		36.696	BB	251636	3698	0.85		

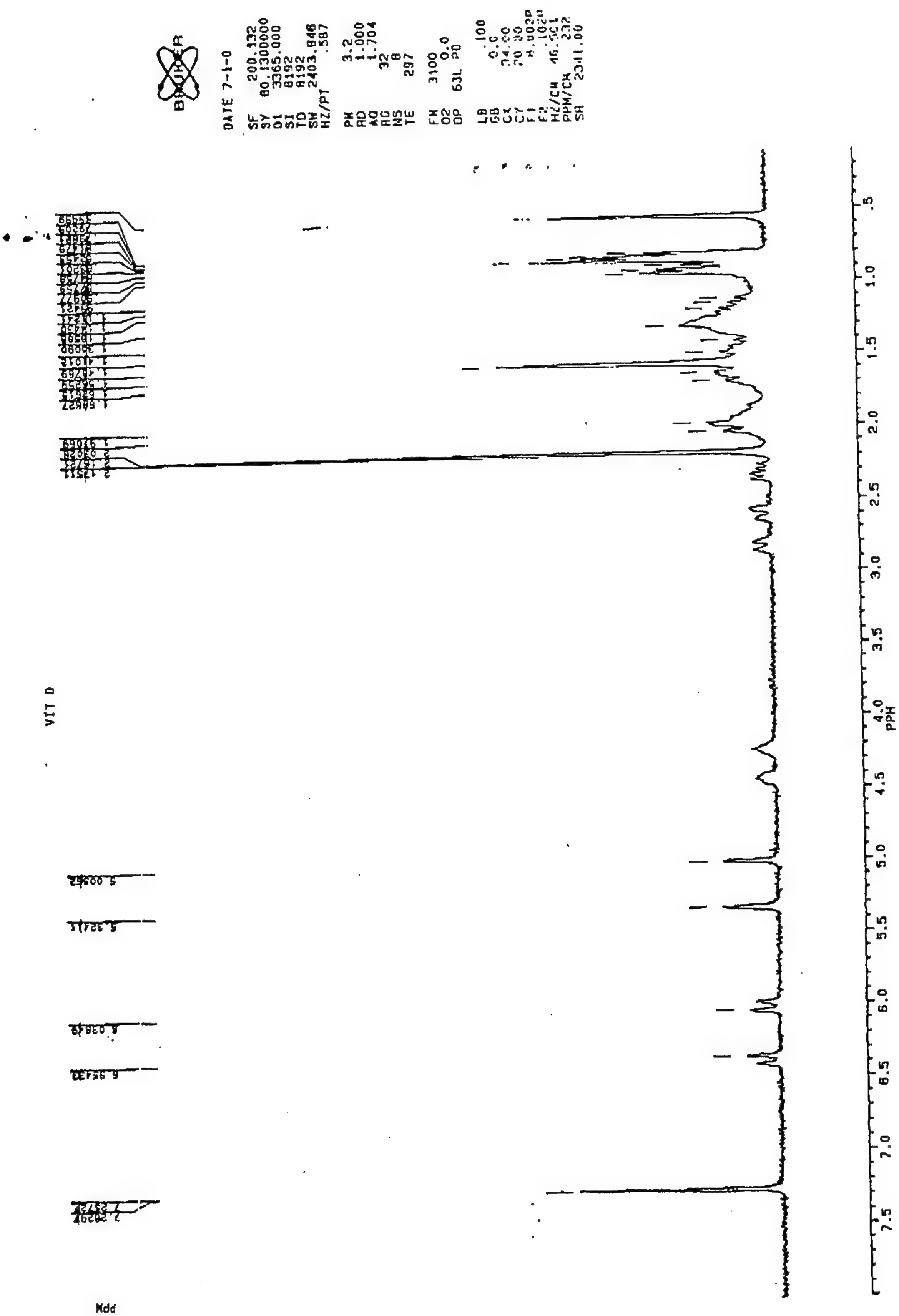
Name: Tania Parri

Notebook Reference: LN 24 pg. 72

Date: 1/5/00



Tania Parr LN 341 pg. 73


VIT D
PPM

SIGMA

Certificate of Analysis

TEST	SPECIFICATION	LOT {107H1649} RESULTS
Product Name	Corn oil	
Product Number	C8267	
CAS Number	8001-30-7	
APPEARANCE	CLEAR YELLOW TO YELLOW-GREEN LIQUID	CLEAR YELLOW LIQUID
FREE FATTY ACIDS *	LESS THAN 2.0 ML OF 0.02 N SODIUM HYDROXIDE REQUIRED TO NEUTRALIZE 10 G OF CORN OIL	0.30 ML OF 0.02 N SODIUM CHLORIDE REQUIRED TO NEUTRALIZE 10.0 G CORN OI
HEAVY METALS *	NOT MORE THAN 0.001% (AS LEAD)	<0.001%
IODINE VALUE *	102 TO 130	127
* SUPPLIER TEST RESUL		
QC ACCEPTANCE DATE		NOVEMBER 1997

David Feldker, Manager
Analytical Services

Appendix C. Individual Animal Data

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-1

Individual Animal Daily Clinical Observations - Males

Dose Group: 1 (Vehicle Control; 0 μg/kg)

Animal Number	Observation	Onset	Duration	Frequency
1252	Normal	Day 1	Day 36	36
	Terminal Sacrifice	Day 37	Day 37	1
1256	Normal	Day 1	Day 36	36
	Terminal Sacrifice	Day 37	Day 37	1
1258	Normal	Day 1	Day 8	8
	Moved[a]	Day 9	Day 9	1
1263	Normal	Day 1	Day 36	35
	Diarrhea	Day 14	Day 14	1
	Terminal Sacrifice	Day 37	Day 37	1
1266	Normal	Day 1	Day 8	8
	Moved[a]	Day 9	Day 9	1

[a] Moved from Group 1 to Group 5 on Day 9

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-1 (cont.)

Individual Animal Daily Clinical Observations - Males

Dose Group: 2 (Low; 10 μg/kg)

Animal Number	Observation	Onset	Duration	Frequency
1257	Normal	Day 1	Day 28	24
	Diarrhea	Day 8	Day 10	3
	Emesis (Bile)	Day 7	Day 7	1
	Terminal Sacrifice	Day 29	Day 29	1
1260	Normal	Day 1	Day 28	26
	Diarrhea	Day 9	Day 15	2
	Terminal Sacrifice	Day 29	Day 29	1
1262	Normal	Day 1	Day 27	27
	Bloody Salivation	Day 28	Day 28	1
	Swollen Cheeks	Day 28	Day 28	1
	Thin	Day 28	Day 28	1
	Terminal Sacrifice	Day 29	Day 29	1

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-1 (cont.)

Individual Animal Daily Clinical Observations - Males

Dose Group: 3 (Mid; 30 μg/kg)

Animal Number	Observation	Onset	Duration	Frequency
1259	Normal	Day 1	Day 21	21
	Bloody Salivation	Day 23	Day 23	1
	Emaciated	Day 22	Day 23	2
	Hypoactive	Day 23	Day 23	1
	Swollen Cheeks	Day 23	Day 23	1
	Found Dead	Day 24	Day 24	1
1261	Normal	Day 1	Day 21	20
	Bloody Salivation	Day 23	Day 24	2
	Cold To Touch	Day 24	Day 24	1
	Diarrhea	Day 20	Day 20	1
	Emaciated	Day 22	Day 24	3
	Hypoactive	Day 24	Day 24	1
	Swollen Cheeks	Day 23	Day 24	2
	Moribund Sacrifice	Day 24	Day 24	1
1265	Normal	Day 1	Day 21	18
	Bloody Salivation	Day 27	Day 27	1
	Diarrhea	Day 10	Day 12	3
	Emaciated	Day 22	Day 28	7
	Hypoactive	Day 24	Day 28	5
	Terminal Sacrifice	Day 29	Day 29	1

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-1 (cont.)

Individual Animal Daily Clinical Observations - Males

Dose Group: 4 (High; 90/45[a] μg/kg)

Animal Number	Observation	Onset	Duration	Frequency
1251	Normal	Day 1	Day 17	17
	Cold to Touch	Day 22	Day 26	5
	Diarrhea	Day 25	Day 26	2
	Emaciated	Day 18	Day 26	9
	Hypoactive	Day 24	Day 26	3
	Found Dead	Day 27	Day 27	1
1253	Normal	Day 1	Day 14	14
	Cold To Touch	Day 22	Day 23	2
	Dehydrated	Day 23	Day 23	1
	Diarrhea	Day 23	Day 23	1
	Emaciated	Day 15	Day 23	9
	Hypoactive	Day 22	Day 23	2
	Labored Breathing	Day 23	Day 23	1
	Moribund Sacrifice	Day 23	Day 23	1
1254	Normal	Day 1	Day 17	16
	Diarrhea	Day 14	Day 14	1
	Emaciated	Day 18	Day 28	11
	Hypoactive	Day 25	Day 29	5
	Thin	Day 29	Day 29	1
	Terminal Sacrifice	Day 30	Day 30	1
1255	Normal	Day 1	Day 14	9
	Cold To Touch	Day 22	Day 22	1
	Diarrhea	Day 6	Day 18	4
	Emaciated	Day 15	Day 22	8
	Hypoactive	Day 8	Day 22	11
	Found Dead	Day 23	Day 23	1
1264	Normal	Day 1	Day 17	14
	Bloody Salivation	Day 25	Day 29	5
	Cold To Touch	Day 29	Day 29	1
	Diarrhea	Day 9	Day 10	2
	Emaciated	Day 18	Day 29	12
	Hypoactive	Day 27	Day 29	3
	Lacrimation	Day 8	Day 8	1
	Ocular Discharge	Day 24	Day 28	5
	Swollen Cheeks	Day 25	Day 29	5
	Terminal Sacrifice	Day 30	Day 30	1

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-1 (cont.)

Individual Animal Daily Clinical Observations - Males

Dose Group: 5 (Low-Low; 5 μg/kg)

Animal Number	Observation	Onset	Duration	Frequency
1258	Normal	Day 1	Day 28	27
	Diarrhea	Day 6	Day 6	1
	Terminal Sacrifice	Day 29	Day 29	1
1266	Normal	Day 1	Day 28	27
	Diarrhea	Day 6	Day 6	1
	Terminal Sacrifice	Day 29	Day 29	1

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-1 (cont.)

Individual Animal Daily Clinical Observations - Females

Dose Group: 1 (Vehicle Control; 0 μg/kg)

Animal Number	Observation	Onset	Duration	Frequency
1235	Normal	Day 1	Day 35	34
	Diarrhea	Day 13	Day 13	1
	Terminal Sacrifice	Day 36	Day 36	1
1236	Normal	Day 1	Day 7	7
	Moved[a]	Day 9	Day 9	1
1244	Normal	Day 1	Day 7	7
	Moved[a]	Day 9	Day 9	1
1245	Normal	Day 1	Day 35	34
	Emesis (Bile)	Day 7	Day 7	1
	Terminal Sacrifice	Day 36	Day 36	1
1249	Normal	Day 1	Day 35	34
	Diarrhea	Day 13	Day 13	1
	Terminal Sacrifice	Day 36	Day 36	1

[a] Moved from Group 1 to Group 5 on Day 8

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-1 (cont.)

Individual Animal Daily Clinical Observations - Females

Dose Group: 2 (Low; 10 μg/kg)

Animal Number	Observation	Onset	Duration	Frequency
1242	Normal	Day 1	Day 27	27
	Thin	Day 28	Day 28	1
	Terminal Sacrifice	Day 29	Day 29	1
1246	Normal	Day 1	Day 27	26
	Diarrhea	Day 14	Day 14	1
	Thin	Day 28	Day 28	1
	Terminal Sacrifice	Day 29	Day 29	1
1250	Normal	Day 1	Day 28	28
	Terminal Sacrifice	Day 29	Day 29	1

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-1 (cont.)

Individual Animal Daily Clinical Observations - Females

Dose Group: 3 (Mid; 30 μg/kg)

Animal Number	Observation	Onset	Duration	Frequency
1238	Normal	Day 1	Day 20	20
	Cold To Touch	Day 27	Day 27	1
	Emaciated	Day 21	Day 28	8
	Hypoactive	Day 22	Day 27	6
	Terminal Sacrifice	Day 29	Day 29	1
1239	Normal	Day 1	Day 20	20
	Cold To Touch	Day 24	Day 27	4
	Emaciated	Day 21	Day 27	7
	Hypoactive	Day 24	Day 27	4
	Moribund Sacrifice	Day 28	Day 28	1
1243	Normal	Day 1	Day 20	18
	Cold To Touch	Day 24	Day 27	4
	Diarrhea	Day 15	Day 15	1
	Emaciated	Day 21	Day 28	8
	Emesis (Bile)	Day 14	Day 14	1
	Hypoactive	Day 25	Day 27	3
	Terminal Sacrifice	Day 29	Day 29	1

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-1 (cont.)

Individual Animal Daily Clinical Observations - Females

Dose Group: 4 (High; 90/45[a] µg/kg)

Animal Number	Observation	Onset	Duration	Frequency
1237	Normal	Day 1	Day 13	13
	Emaciated	Day 14	Day 28	15
	Thin	Day 29	Day 29	1
	Terminal Sacrifice	Day 30	Day 30	1
1240	Normal	Day 1	Day 16	8
	Diarrhea	Day 6	Day 13	6
	Emaciated	Day 17	Day 29	12
	Emesis (Bile)	Day 7	Day 29	3
	Thin	Day 28	Day 28	1
	Terminal Sacrifice	Day 30	Day 30	1
1241	Normal	Day 1	Day 16	14
	Diarrhea	Day 9	Day 9	1
	Emaciated	Day 17	Day 29	12
	Emesis (Bile)	Day 7	Day 7	1
	Thin	Day 28	Day 28	1
	Terminal Sacrifice	Day 30	Day 30	1
1247	Normal	Day 1	Day 5	5
	Emesis (Bile)	Day 6	Day 6	1
	Found Dead	Day 7	Day 7	1
1248	Normal	Day 1	Day 5	5
	Found Dead	Day 6	Day 6	1

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-1 (cont.)

Individual Animal Daily Clinical Observations - Females

Dose Group: 5 (Low-Low; 5 μg/kg)

Animal Number	Observation	Onset	Duration	Frequency
1236	Normal	Day 1	Day 28	28
	Terminal Sacrifice	Day 29	Day 29	1
1244	Normal	Day 1	Day 28	28
	Terminal Sacrifice	Day 29	Day 29	1

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-2

Individual Animal Body Weights (kg)

Males

Animal Number	Group	Dose (μg/kg)	Day 1[a]	Day 8	Day 15	Day 22	Day 29
1252	1 (VCTL)	0	7.24	7.74	7.84	8.34	8.26
1256		0	7.60	7.80	7.92	8.48	8.46
1258		0	7.02	7.34	Moved[b]	--	--
1263		0	7.14	7.60	7.76	7.96	8.12
1266		0	8.24	9.10	Moved[b]	--	--
1257	2	10	6.94	6.26	5.70	5.24	4.86
1260		10	6.92	7.10	6.92	6.42	5.78
1262		10	6.80	6.88	6.06	5.34	4.46
1259	3	30	6.80	6.38	5.34	4.54	Dead
1261		30	7.94	6.78	5.84	4.94	Dead
1265		30	7.68	6.94	6.02	5.40	4.96
1251	4	90/45[c]	7.64	6.80	6.04	5.08	Dead
1253		90/45	7.00	6.14	5.30	4.72	Dead
1254		90/45	7.64	6.56	5.88	5.50	4.96
1255		90/45	6.78	5.92	5.12	4.44	Dead
1264		90/45	7.32	6.18	5.46	5.00	4.46
1252	1 (VCTL)	0	7.74	7.84	8.34	8.26	8.60
1256		0	7.80	7.92	8.48	8.46	8.70
1263		0	7.60	7.76	7.96	8.12	8.52
1258	5	5	7.34	7.32	7.84	8.02	8.18
1266		5	9.10	9.14	9.34	9.40	8.98

[a] predose
[b] animal switched to 5 μg/kg dose group (Group 5)
[c] dose decreased from 90 to 45 μg/kg on Day 9

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-2

Individual Animal Body Weights (kg)

Females

Animal Number	Group	Dose (μg/kg)	Day 1[a]	Day 8	Day 15	Day 22	Day 29
1235	1 (VCTL)	0	7.16	6.84	6.72	7.20	7.48
1236		0	6.34	6.48	Moved[b]	--	--
1244		0	6.76	6.78	Moved[b]	--	--
1245		0	7.16	7.12	7.00	7.30	7.78
1249		0	7.40	7.82	7.98	8.48	9.06
1242	2	10	5.80	5.34	5.02	4.64	4.32
1246		10	6.74	6.20	5.90	5.60	5.02
1250		10	7.00	6.88	6.48	5.94	5.38
1238	3	30	6.86	5.84	5.08	4.54	4.04
1239		30	6.78	5.56	4.80	4.30	Dead
1243		30	6.70	5.64	4.84	4.16	3.64
1237	4	90/45[c]	6.94	5.62	4.92	4.68	4.70
1240		90/45	7.20	5.90	4.86	4.36	4.10
1241		90/45	6.82	5.50	4.84	4.50	4.16
1247		90/45	6.68	Dead	--	--	--
1248		90/45	7.74	Dead	--	--	--
1235	1 (VCTL)	0	6.84	6.72	7.20	7.48	7.40
1245		0	7.12	7.00	7.30	7.78	7.64
1249		0	7.82	7.98	8.48	9.06	8.84
1236	5	5	6.48	6.34	6.66	6.92	6.68
1244		5	6.78	6.78	7.12	7.36	7.26

[a] predose
[b] animal switched to 5 μg/kg dose group (Group 5)
[c] dose decreased from 90 to 45 μg/kg on Day 8

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-3

Individual Animal Body Weight Gains (kg)

Males

Animal Number	Group	Dose (μg/kg)	Day 8	Day 15	Day 22	Day 29	Total
1252	1 (VCTL)	0	0.50	0.10	0.50	-0.08	1.02
1256		0	0.20	0.12	0.56	-0.02	0.86
1258		0	0.32	Moved[a]	--	--	--
1263		0	0.46	0.16	0.20	0.16	0.98
1266		0	0.86	Moved[a]	--	--	--
1257	2	10	-0.68	-0.56	-0.46	-0.38	-2.08
1260		10	0.18	-0.18	-0.50	-0.64	-1.14
1262		10	0.08	-0.82	-0.72	-0.88	-2.34
1259	2	30	-0.42	-1.04	-0.80	Dead	--
1261		30	-1.16	-0.94	-0.90	Dead	--
1265		30	-0.74	-0.92	-0.62	-0.44	-2.72
1251	4	90/45[b]	-0.84	-0.76	-0.96	Dead	--
1253		90	-0.86	-0.84	-0.58	Dead	--
1254		90	-1.08	-0.68	-0.38	-0.54	-2.68
1255		90	-0.86	-0.80	-0.68	Dead	--
1264		90	-1.14	-0.72	-0.46	-0.54	-2.86
1252	1 (VCTL)	0	0.10	0.50	-0.08	0.34	0.86
1256		0	0.12	0.56	-0.02	0.24	0.90
1263		0	0.16	0.20	0.16	0.40	0.92
1258	5	5	-0.02	0.52	0.18	0.16	0.84
1266		5	0.04	0.20	0.06	-0.42	-0.12

[a] animal switched to 5 μg/kg dose group (Group 5)
[b] dose decreased from 90 to 45 μg/kg on Day 9

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-3 (cont.)

Individual Animal Body Weight Gains (kg)

Females

Animal Number	Group	Dose (µg/kg)	Day 8	Day 15	Day 22	Day 29	Total
1235	1 (VCTL)	0	-0.12	0.48	0.28	-0.08	0.56
1236		0	0.14	Moved[a]	--	--	--
1244		0	0.02	Moved[a]	--	--	--
1245		0	-0.04	-0.12	0.30	0.48	0.62
1249		0	0.42	0.16	0.50	0.58	1.66
1242	2	10	-0.46	-0.32	-0.38	-0.32	-1.48
1246		10	-0.54	-0.30	-0.30	-0.58	-1.72
1250		10	-0.12	-0.40	-0.54	-0.56	-1.62
1238	3	30	-1.02	-0.76	-0.54	-0.50	-2.82
1239		30	-1.22	-0.76	-0.50	Dead	--
1243		30	-1.06	-0.80	-0.68	-0.52	-3.06
1237	4	90/45[b]	-1.32	-0.70	-0.24	0.02	-2.24
1240		90	-1.30	-1.04	-0.50	-0.26	-3.10
1241		90	-1.32	-0.66	-0.34	-0.34	-2.66
1247		90	Dead	--	--	--	--
1248		90	Dead	--	--	--	--

1235	1 (VCTL)	0	-0.12	0.48	0.28	-0.08	0.56
1245		0	-0.12	0.30	0.48	-0.14	0.52
1249		0	0.16	0.50	0.58	-0.22	1.02
1236	5	5	-0.14	0.32	0.26	-0.24	0.20
1244		5	0.00	0.34	0.24	-0.10	0.48

[a] animal switched to 5 µg/kg dose group (Group 5)
[b] dose decreased from 90 to 45 µg/kg on Day 8

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-4

Individual Animal Daily Food Consumption (g) - Males

Animal Number	Dose (μg/kg)	Day 1	2	3	4	5	6	7	8	9
1252	0	251	170	214	183	229	196	144	260	145
1256	0	65	128	209	167	239	291	280	300	150
1258	0	300	129	161	192	213	190	134	203	Moved[a]
1263	0	190	156	214	192	224	152	300	300	169
1266	0	300	247	207	270	300	271	282	104	Moved[a]
1257	10	7	135	49	123	87	92	140	231	30
1260	10	235	139	121	148	192	124	300	300	162
1262	10	248	238	149	204	220	188	100	134	27
1259	30	279	158	168	158	75	61	58	0	0
1261	30	165	149	161	228	116	125	63	33	1
1265	30	200	138	162	123	68	92	119	55	0
1251	90/45[b]	253	111	124	98	21	26	23	23	47
1253	90/45	300	123	88	119	0	0	69	0	17
1254	90/45	273	130	42	61	0	0	85	12	18
1255	90/45	246	132	126	60	0	0	0	0	31
1264	90/45	147	112	95	82	60	20	0	0	25
1252	0	145	193	208	186	244	259	229	216	236
1256	0	150	209	227	158	277	284	300	300	222
1263	0	169	177	234	217	274	263	285	228	226
1258	5	125	256	183	181	256	247	239	292	200
1266	5	230	253	245	222	300	300	292	300	282

[a] animal switched to 5 μg/kg dose group (Group 5)
[b] dose decreased from 90 to 45 μg/kg on Day 9

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-4 (cont.)

Individual Animal Daily Food Consumption (g)

Males

Animal Number	Dose (µg/kg)	Day 10	11	12	13	14	15	16	17	18
1252	0	193	208	186	244	259	229	216	236	293
1256	0	209	227	158	277	284	300	300	222	300
1258	0	Moved[a]	--	--	--	--	--	--	--	--
1263	0	177	234	217	274	263	285	228	226	300
1266	0	Moved[a]	--	--	--	--	--	--	--	--
1257	10	63	89	49	27	18	79	49	23	36
1260	10	169	243	138	229	209	91	141	128	120
1262	10	36	48	32	42	97	77	21	15	53
1259	30	19	0	0	0	6	0	0	0	12
1261	30	34	25	0	0	0	14	14	35	3
1265	30	39	0	4	0	11	15	0	14	17
1251	90/45[b]	7	41	0	9	69	50	6	42	15
1253	90/45	0	23	0	56	20	19	58	50	18
1254	90/45	30	0	3	53	33	76	22	95	33
1255	90/45	4	0	0	0	17	0	22	2	25
1264	90/45	21	0	18	21	0	11	40	0	39
1252	0	293	283	294	300	300	219	247	166	300
1256	0	300	300	300	300	300	245	300	300	300
1263	0	300	300	300	300	300	286	286	177	300
1258	5	282	300	295	300	300	300	228	241	300
1266	5	300	300	300	300	294	202	300	218	300

[a] animal switched to 5 µg/kg dose group (Group 5)
[b] dose decreased from 90 to 45 µg/kg on Day 9

Appendix C Table C-4 (cont.)

Individual Animal Daily Food Consumption (g)

Males

Animal Number	Dose (µg/kg)	Day 19	20	21	22	23	24	25	26	27	28
1252	0	283	294	300	300	219	247	166	300	300	296
1256	0	300	300	300	300	245	300	300	300	239	300
1258	0	Moved[a]	--	--	--	--	--	--	--	--	--
1263	0	300	300	300	300	286	286	177	300	300	300
1266	0	Moved[a]	--	--	--	--	--	--	--	--	--
1257	10	40	40	67	37	17	22	30	32	19	65
1260	10	126	81	98	120	78	51	13	87	18	74
1262	10	44	42	51	48	29	43	53	81	69	110
1259	30	0	0	0	0	0	Dead	--	--	--	--
1261	30	22	16	28	2		Dead	--	--	--	--
1265	30	21	17	20	53	0	28	2	19	2	6
1251	90/45[b]	6	12	16	2	6	1	16	0	Dead	--
1253	90/45	102	28	116	0	Dead	--	--	--	--	--
1254	90/45	29	132	53	7	74	94	32	156	0	143
1255	90/45	23	48	51	18	Dead	--	--	--	--	—
1264	90/45	53	25	51	41	0	19	8	15	46	34
1252	0	300	296	300	300	300	300	129	188	300	300
1256	0	239	300	300	244	300	300	122	177	300	300
1263	0	300	300	300	300	300	300	163	185	300	300
1258	5	290	300	300	277	300	300	67	154	300	300
1266	5	300	300	300	292	300	300	181	227	300	300

[a] animal switched to 5 µg/kg dose group (Group 5)
[b] dose decreased from 90 to 45 µg/kg on Day 9

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-4 (cont.)

Individual Animal Daily Food Consumption (g)

Females

Animal Number	Dose (µg/kg)	Day 1	2	3		5	6	7	8	9
1235	0	51	71	134		155	[illegible]	300	**169**	165
1236	0	134	185	165	[illegible]	[illegible]	290	300	**Moved**[a]	--
1244	0	157	155	171	[illegible]		164	252	**Moved**[a]	--
1245	0	97	136	147	201	180	168	242	**133**	**180**
1249	0	213	192	173	253	225	300	71	**250**	**217**
1242	10	133	99	90	208	[illegible]	[illegible]	187	**104**	165
1246	10	105	171	111	140	128	[illegible]	300	**83**	**94**
1250	10	190	103	106	173	134	194	213	**102**	**92**
1238	30	0	57	65	[illegible]	17	42	19	**8**	**4**
1239	30	141	128	77			[illegible]	86	13	14
~~1243~~	30	154	133	128	105		95	70	0	0
1237	90/45[b]	66	126	58	14	[illegible]	19	0	17	4
~~1240~~	~~90~~/45	165	**98**	94	**81**	[illegible]	0	0	0	0
1241	90/45	**98**	132	74	[illegible]	71	36	0	22	23
1247	90/45	**160**	116	84		0	0	Dead	--	--
1248	90/45	104	179	20		0	Dead	--	--	--

1235	0	169	165	197	195	249	233	161	239	209
1245	0	133	180	196	209	240	227	181	228	186
1249	0	250	217	300	283	300	291	222	277	261
1236	5	174	199	300	239	300	300	190	240	219
1244	5	64	178	205	194	300	282	167	292	191

[a] animal switched to 5 µg/kg dose group (Group 5)
[b] dose decreased from 90 to 45 µg/kg on Day 8

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FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-4 (cont.)

Individual Animal Daily Food Consumption (g)

Females

Animal Number	Dose (μg/kg)	10	11	12	13	14	15	16	17	18
1235	0	197	195	249	233	161	239	209	260	243
1236	0	Moved[a]	--	--	--	--	--	--	--	--
1244	0	Moved[a]	--	--	--	--	--	--	--	--
1245	0	196	209	240	227	181	228	186	248	227
1249	0	300	283	300	291	222	277	261	300	300
1242	10	73	106	109	103	73	65	52	100	64
1246	10	107	133	193	174	132	194	112	133	170
1250	10	131	139	141	139	169	99	79	79	49
1238	30	7	0	0	24	11	0	7	1	0
1239	30	1	12	13	11	7	19	8	30	29
1243	30	2	1	7	10	1	10	14	24	14
1237	90/45[b]	0	6	17	15	45	17	23	42	65
1240	90/45	1	0	8	24	3	5	6	14	15
1241	90/45	20	38	N/D[c]	N/D	14	10	42	15	18
1247	90/45	Dead	--	--	--	--	--	--	--	--
1248	90/45	Dead	--	--	--	--	--	--	--	--

1235	0	260	243	291	300	300	199	206	125	300
1245	0	248	227	257	300	300	228	234	184	300
1249	0	300	300	300	300	300	300	300	300	300
1236	5	300	289	300	300	300	181	228	114	300
1244	5	296	248	300	300	300	213	247	154	300

[a] animal switched to 5 μg/kg dose group (Group 5)
[b] dose decreased from 90 to 45 μg/kg on Day 8
[c] N/D = not done; no data

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-4 (cont.)

Individual Animal Daily Food Consumption (g)

Females

Animal Number	Dose (µg/kg)	Day 19	20	21	22	23	24	25	26	27	28
1235	0	291	300	300	199	206	125	300	256	300	300
1236	0	Moved[a]	--	--	--	--	--	--	--	--	--
1244	0	Moved[a]	--	--	--	--	--	--	--	--	--
1245	0	257	300	300	228	234	184	300	300	300	300
1249	0	300	300	300	300	300	300	300	300	300	300
1242	10	54	120	49	11	50	63	42	29	46	31
1246	10	116	111	37	21	28	50	35	39	2	17
1250	10	69	103	62	17	39	22	33	43	41	19
1238	30	7	52	3	4	6	7	5	2	7	0
1239	30	33	28	14	16	14	37	30	20	7	Dead
1243	30	19	11	10	5	10	5	60	6	13	2
1237	90/45[b]	30	115	25	57	110	43	177	59	161	122
1240	90/45	24	21	25	15	15	8	32	8	16	36
1241	90/45	33	22	4	87	94	20	59	0	15	37
1247	90/45	Dead	--	--	--	--	--	--	--	--	--
1248	90/45	Dead	--	--	--	--	--	--	--	--	--
1235	0	256	300	300	230	300	300	55	125	300	300
1245	0	300	300	300	300	300	300	300	206	300	300
1249	0	300	300	300	300	300	300	300	300	300	300
1236	5	300	279	300	300	300	300	71	132	300	300
1244	5	233	217	300	229	300	300	18	144	300	300

[a] animal switched to 5 µg/kg dose group (Group 5)
[b] dose decreased from 90 to 45 µg/kg on Day 8

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-5

Individual Animal Hematology Data - Males

Pre-test

Animal Number	Group	Dose (µg/kg)	WBC thsn/cmm	RBC mill/cmm	HGB g/dL	HCT %	MCV fl	MCH pg	MCHC %	PLT thsn/cmm	RETPC %
1252	1 (VCTL)	0	12.6	6.71	15.0	45.2	67.3	22.4	33.2	497	1.1
1256	1 (VCTL)	0	7.5	6.51	14.5	42.9	65.9	22.3	33.8	412	1.7
1258	1 (VCTL)	0	14.6	6.57	14.6	44.2	67.2	22.2	33.0	379	1.8
1263	1 (VCTL)	0	23.8	6.97	15.5	46.5	66.7	22.2	33.3	492	0.9
1266	1 (VCTL)	0	13.8	6.60	15.5	46.1	69.8	23.5	33.6	422	2.0
1257	2	10	12.3	5.81	13.5	40.0	68.9	23.2	33.8	410	1.0
1260	2	10	14.8	6.26	14.8	43.8	70.0	23.6	33.8	436	0.8
1262	2	10	22.1	7.30	15.7	47.2	64.7	21.5	33.3	377	2.3
1259	3	30	11.0	6.88	16.7	50.2	72.9	24.3	33.3	347	3.2
1261	3	30	14.0	6.83	14.8	44.9	65.8	21.7	33.0	464	2.3
1265	3	30	20.3	6.52	15.2	46.4	71.2	23.3	32.8	433	2.7
1251	4	90	9.7	6.38	15.0	45.4	71.1	23.5	33.0	486	1.0
1253	4	90	14.5	6.17	14.1	42.3	68.5	22.9	33.3	544	2.4
1254	4	90	16.9	6.20	14.2	43.2	69.6	22.9	32.9	430	2.1
1255	4	90	14.1	6.93	14.8	45.3	65.3	21.4	32.7	398	1.4
1264	4	90	15.9	6.88	16.2	48.7	70.8	23.5	33.3	355	1.4

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-5 (cont.)

Individual Animal Hematology Data - Males

Pre-test

Animal Number	Group	Dose (µg/kg)	RETABS thsn/cmm	NRBC #/100 WBC	SEG NEU thsn/cmm	BAND NEU thsn/cmm	LYMPH thsn/cmm	MONO thsn/cmm	EOSIN thsn/cmm	BASO thsn/cmm
1252	1 (VCTL)	0	73.8	0	9.6	0.3	2.0	0.3	0.5	0.0
1256	1 (VCTL)	0	110.7	0	5.6	0.0	1.4	0.5	0.1	0.0
1258	1 (VCTL)	0	62.7	0	18.3	1.2	3.1	1.2	0.0	0.0
1263	1 (VCTL)	0	118.3	0	8.6	0.3	4.2	1.0	0.4	0.0
1266	1 (VCTL)	0	132.0	0	9.1	0.0	3.6	1.0	0.1	0.0
1257	2	10	58.1	0	9.2	0.2	2.5	0.4	0.0	0.0
1260	2	10	50.1	0	10.8	0.6	2.8	0.3	0.3	0.0
1262	2	10	167.9	0	16.4	0.4	4.0	1.1	0.2	0.0
1259	3	30	220.2	0	7.0	0.1	2.3	1.3	0.2	0.0
1261	3	30	157.1	0	10.6	0.0	2.9	0.4	0.0	0.0
1265	3	30	176.0	0	14.0	0.2	5.1	0.8	0.2	0.0
1251	4	90	63.8	0	6.3	0.2	2.5	0.6	0.1	0.0
1253	4	90	148.1	0	11.2	0.4	1.7	0.9	0.3	0.0
1254	4	90	130.2	1	12.0	0.0	3.4	1.4	0.2	0.0
1255	4	90	97.0	0	10.0	0.3	3.2	0.4	0.1	0.0
1264	4	90	96.3	0	11.8	0.0	3.0	1.0	0.2	0.0

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-5 (cont.)

Individual Animal Hematology Data - Males

Pre-test

Animal Number	Group	Dose (µg/kg)	NRBC #/100 WBC	SEG NEU %	BAND NEU %	LYMPH %	MONO %	EOSIN %	BASO %
1252	1 (VCTL)	0	0	76	2	16	2	4	0
1256	1 (VCTL)	0	0	75	0	18	6	1	0
1258	1 (VCTL)	0	0	77	5	13	5	0	0
1263	1 (VCTL)	0	0	59	2	29	7	3	0
1266	1 (VCTL)	0	0	66	0	26	7	1	0
1257	2	10	0	75	2	20	3	0	0
1260	2	10	0	73	4	19	2	2	0
1262	2	10	0	74	2	18	5	1	0
1259	3	30	0	64	1	21	12	2	0
1261	3	30	0	76	0	21	3	0	0
1265	3	30	0	69	1	25	4	1	0
1251	4	90	0	65	2	26	6	1	0
1253	4	90	0	77	3	12	6	2	0
1254	4	90	1	71	0	20	8	1	0
1255	4	90	0	71	2	23	3	1	0
1264	4	90	0	74	0	19	6	1	0

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-5 (cont.)

Individual Animal Hematology Data - Females

Pre-test

Animal Number	Group	Dose (μg/kg)	WBC thsn/cmm	RBC mill/cmm	HGB g/dL	HCT %	MCV fl	MCH pg	MCHC %	PLT thsn/cmm	RETPC %
1235	1 (VCTL)	0	9.5	7.18	16.4	49.1	68.4	22.8	33.4	386	2.1
1236	1 (VCTL)	0	9.0	7.20	15.9	48.7	67.6	22.1	32.6	411	2.0
1244	1 (VCTL)	0	12.4	7.05	16.6	49.4	70.0	23.5	33.6	470	1.3
1245	1 (VCTL)	0	10.0	6.42	15.9	47.1	73.4	24.8	33.8	420	2.2
1249	1 (VCTL)	0	12.5	6.52	13.8	40.4	62.0	21.2	34.2	506	1.1
1242	2	10	16.9	6.29	14.5	43.8	69.7	23.1	33.1	340	1.6
1246	2	10	12.4	6.94	16.3	48.0	69.1	23.5	34.0	378	2.5
1250	2	10	11.6	6.75	15.7	47.3	70.1	23.3	33.2	381	2.4
1238	3	30	7.6	7.11	16.5	49.3	69.3	23.2	33.5	376	1.8
1239	3	30	9.9	6.50	14.9	45.3	69.7	22.9	32.9	367	0.9
1243	3	30	12.9	5.78	13.8	39.7	68.6	23.9	34.8	384	2.1
1237	4	90	14.2	6.45	15.2	45.7	70.8	23.6	33.3	369	1.3
1240	4	90	14.2	6.44	14.9	44.1	68.5	23.1	33.8	449	1.2
1241	4	90	13.6	6.39	15.3	44.5	69.6	23.9	34.4	474	0.6
1247	4	90	12.6	7.02	15.5	46.3	66.0	22.1	33.5	360	1.3
1248	4	90	15.4	6.81	15.4	45.5	66.8	22.6	33.8	618	1.9

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-5 (cont.)

Individual Animal Hematology Data - Females

Pre-test

Animal Number	Group	Dose (µg/kg)	RETABS thsn/cmm	NRBC #/100 WBC	SEG NEU thsn/cmm	BAND NEU thsn/cmm	LYMPH thsn/cmm	MONO thsn/cmm	EOSIN thsn/cmm	BASO thsn/cmm
1235	1 (VCTL)	0	150.8	1	5.5	0.1	3.1	0.6	0.2	0.0
1236	1 (VCTL)	0	144.0	0	6.0	0.0	2.9	0.0	0.1	0.0
1244	1 (VCTL)	0	91.7	0	8.2	0.0	3.5	0.6	0.1	0.0
1245	1 (VCTL)	0	141.2	0	5.5	0.1	3.4	0.9	0.1	0.0
1249	1 (VCTL)	0	71.7	0	9.5	0.3	2.4	0.4	0.0	0.0
1242	2	10	100.6	0	11.7	0.8	3.0	1.4	0.0	0.0
1246	2	10	173.5	0	8.4	0.1	2.9	0.9	0.1	0.0
1250	2	10	162.0	0	6.6	0.1	4.2	0.5	0.2	0.0
1238	3	30	128.0	0	4.8	0.2	2.1	0.5	0.2	0.0
1239	3	30	58.5	0	6.3	0.1	2.8	0.5	0.2	0.0
1243	3	30	121.4	0	9.4	0.4	2.6	0.4	0.1	0.0
1237	4	90	83.8	0	10.8	0.0	2.4	0.9	0.1	0.0
1240	4	90	77.3	0	9.2	0.0	4.0	0.7	0.3	0.0
1241	4	90	38.3	0	9.7	0.1	2.9	0.7	0.3	0.0
1247	4	90	91.3	0	8.9	0.0	3.3	0.4	0.0	0.0
1248	4	90	129.4	0	10.5	0.0	4.0	0.8	0.2	0.0

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-5 (cont.)

Individual Animal Hematology Data - Females

Pre-test

Animal Number	Group	Dose (µg/kg)	NRBC #/100 WBC	SEG NEU %	BAND NEU %	LYMPH %	MONO %	EOSIN %	BASO %
1252	1 (VCTL)	0	1	58	1	33	6	2	0
1256	1 (VCTL)	0	0	67	0	32	0	1	0
1258	1 (VCTL)	0	0	66	0	28	5	1	0
1263	1 (VCTL)	0	0	55	1	34	9	1	0
1266	1 (VCTL)	0	0	76	2	19	3	0	0
1257	2	10	0	69	5	18	8	0	0
1260	2	10	0	68	1	23	7	1	0
1262	2	10	0	57	1	36	4	2	0
1259	3	30	0	63	2	27	6	2	0
1261	3	30	0	64	1	28	5	2	0
1265	3	30	0	73	3	20	3	1	0
			0	76	0	17	6	1	0
1251	4	90							
1253	4	90	0	65	0	28	5	2	0
1254	4	90	0	71	1	21	5	2	0
1255	4	90	0	71	0	26	3	0	0
1264	4	90	0	68	0	26	5	1	0

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-6

Individual Animal Hematology Data - Males

Post-dose

Animal Number	Group	Dose (μg/kg)	WBC thsn/cmm	RBC mill/cmm	HGB g/dL	HCT %	MCV fl	MCH pg	MCHC %	PLT thsn/cmm	RETPC %
1252	1 (VCTL)	0	11.0	6.78	15.1	46.0	67.8	22.3	32.8	361	1.0
1256	1 (VCTL)	0	8.9	6.33	14.0	41.9	66.2	22.1	33.4	343	1.1
1263	1 (VCTL)	0	18.6	5.97	13.4	40.2	67.3	22.4	33.3	316	1.4
1257	2	10	7.2	8.33	18.4	57.3	68.8	22.1	32.1	265	1.0
1260	2	10	11.9	7.04	16.6	49.1	69.7	23.6	33.8	386	0.6
1262	2	10	9.9	9.66	19.8	62.3	64.5	20.5	31.8	258	0.9
1259	3	30	Dead	--	--	--	--	--	--	--	--
1261	3	30	21.6	10.08	21.4	66.5	66.0	21.2	32.2	424	0.5
1265	3	30	13.8	7.98	18.2	55.9	70.0	22.8	32.6	304	1.2
1251	4	90/45[a]	12.5	8.45	19.7	60.8	72.0	23.3	32.4	469	0.0
1253	4	90/45	5.5	10.20	22.9	69.4	68.0	22.5	33.0	202	0.0
1254	4	90/45	13.2	8.18	18.8	57.6	70.4	23.0	32.6	328	0.2
1255	4	90/45	Dead	--	--	--	--	--	--	--	--
1264	4	90/45	18.5	7.98	18.9	56.2	70.4	23.7	33.6	212	0.1
1252	1 (VCTL)	0	19.5	6.70	15.3	45.8	68.4	22.8	33.4	348	1.6
1256	1 (VCTL)	0	10.9	6.72	14.6	44.4	66.0	21.7	32.9	311	1.0
1263	1 (VCTL)	0	15.8	6.63	15.0	44.9	67.7	22.6	33.4	358	0.9
1258	5	5	9.8	6.51	14.6	42.9	65.9	22.4	34.0	206	0.9
1266	5	5	29.6	6.96	16.0	48.0	69.0	23.0	33.3	312	1.5

[a] dose decreased from 90 to 45 μg/kg on Day 9

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-6 (cont.)

Individual Animal Hematology Data - Males

Post-dose

Animal Number	Group	Dose (µg/kg)	RETABS thsn/cmm	NRBC #/100 WBC	SEG NEU thsn/cmm	BAND NEU thsn/cmm	LYMPH thsn/cmm	MONO thsn/cmm	EOSIN thsn/cmm	BASO thsn/cmm
1252	1 (VCTL)	0	67.8	0.0	7.0	0.2	2.8	0.7	0.3	0.0
1256	1 (VCTL)	0	69.6	0.0	7.0	0.4	1.3	0.2	0.0	0.0
1263	1 (VCTL)	0	83.6	0.0	13.4	0.6	3.5	0.6	0.6	0.0
1257	2	10	83.3	0.0	4.7	0.1	2.1	0.3	0.0	0.0
1260	2	10	42.2	0.0	9.9	0.2	1.4	0.4	0.0	0.0
1262	2	10	86.9	0.0	8.0	0.1	1.3	0.5	0.0	0.0
1259	3	30	Dead	--	--	--	--	--	--	--
1261	3	30	50.4	0.0	10.6	6.5	1.7	2.6	0.2	0.0
1265	3	30	95.8	0.0	10.2	0.1	2.8	0.7	0.0	0.0
1251	4	90/45[a]	0.0	0.0	9.5	0.6	1.6	0.8	0.0	0.0
1253	4	90/45	0.0	0.0	3.2	0.7	1.4	0.1	0.0	0.0
1254	4	90/45	16.4	0.0	8.2	1.3	2.1	1.6	0.0	0.0
1255	4	90/45	Dead	--	--	--	--	--	--	--
1264	4	90/45	8.0	0.0	14.2	1.1	2.0	0.9	0.2	0.0
1252	1 (VCTL)	0	107.2	0.0	14.6	0.0	3.3	1.2	0.4	0.0
1256	1 (VCTL)	0	67.2	0.0	7.3	0.1	2.7	0.8	0.0	0.0
1263	1 (VCTL)	0	59.7	0.0	10.0	0.2	4.6	0.5	0.6	0.0
1258	5	5	58.6	1.0	6.0	0.0	3.4	0.2	0.2	0.0
1266	5	5	104.4	0.0	16.0	6.5	4.7	2.4	0.0	0.0

[a] dose decreased from 90 to 45 µg/kg on Day 9

Appendix C Table C-6 (cont.)

Individual Animal Hematology Data - Males

Post-dose

Animal Number	Group	Dose (µg/kg)	NRBC #/100 WBC	SEG NEU %	BAND NEU %	LYMPH %	MONO %	EOSIN %	BASO %
1252	1 (VCTL)	0	0	64	2	25	6	3	0
1256	1 (VCTL)	0	0	79	4	15	2	0	0
1263	1 (VCTL)	0	0	72	3	19	3	3	0
1257	2	10	0	65	2	29	4	0	0
1260	2	10	0	83	2	12	3	0	0
1262	2	10	0	81	1	13	5	0	0
1259	3	30	Dead	--	--	--	--	--	--
1261	3	30	0	49	30	8	12	1	0
1265	3	30	0	74	1	20	5	0	0
1251	4	90/45[a]	0	76	5	13	6	0	0
1253	4	90/45	0	59	13	26	2	0	0
1254	4	90/45	0	62	10	16	12	0	0
1255	4	90/45	Dead	--	--	--	--	--	--
1264	4	90/45	0	77	5	11	5	1	0
1252	1 (VCTL)	0	0	75	0	17	6	2	0
1256	1 (VCTL)	0	0	67	1	25	7	0	0
1263	1 (VCTL)	0	0	63	1	29	3	4	0
1258	5	5	1	61	0	35	2	2	0
1266	5	5	0	54	22	16	8	0	0

[a] dose decreased from 90 to 45 µg/kg on Day 9

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-6 (cont.)

Individual Animal Hematology Data - Females

Post-dose

Animal Number	Group	Dose (µg/kg)	WBC thsn/cmm	RBC mill/cmm	HGB g/dL	HCT %	MCV fl	MCH pg	MCHC %	PLT thsn/cmm	RETPC %
1235	1 (VCTL)	0	8.5	6.94	16.0	47.6	68.6	23.1	33.6	314	1.4
1245	1 (VCTL)	0	10.1	6.30	15.4	45.9	72.9	24.4	33.6	361	1.2
1249	1 (VCTL)	0	9.7	6.35	13.2	39.8	62.7	20.8	33.2	352	0.9
1242	2	10	6.4	7.63	17.5	53.7	70.4	22.9	32.6	272	0.9
1246	2	10	9.0	7.49	17.4	51.9	69.3	23.2	33.5	354	0.6
1250	2	10	7.3	7.91	18.4	56.3	71.2	23.3	32.7	304	0.7
1238	3	30	6.2	9.07	20.5	62.1	68.5	22.6	33.0	364	0.8
1239	3	30	9.9	8.58	20.1	60.2	70.2	23.4	33.4	372	0.2
1243	3	30	10.0	8.95	20.1	60.8	67.9	22.5	33.1	460	0.4
1237	4	90/45[a]	14.0	7.47	18.2	53.5	71.6	24.4	34.0	339	0.1
1240	4	90/45	8.9	9.07	20.1	61.2	67.5	22.2	32.8	319	1.2
1241	4	90/45	10.1	7.74	18.6	54.5	70.4	24.0	34.1	480	0.4
1247	4	90/45	Dead	--	--	--	--	--	--	--	--
1248	4	90/45	Dead	--	--	--	--	--	--	--	--
1235	1 (VCTL)	0	15.0	7.05	16.4	48.7	69.1	23.3	33.7	329	1.0
1245	1 (VCTL)	0	14.8	6.50	15.4	47.0	72.3	23.7	32.8	411	2.3
1249	1 (VCTL)	0	14.7	6.51	13.8	41.6	63.9	21.2	33.2	415	1.8
1236	5	5	14.1	7.44	17.0	50.7	68.1	22.8	33.5	338	0.8
1244	5	5	12.2	6.67	15.5	46.3	69.4	23.2	33.5	352	1.3

[a] dose decreased from 90 to 45 µg/kg on Day 8

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-6 (cont.)

Individual Animal Hematology Data - Females

Post-dose

Animal Number	Group	Dose (µg/kg)	RETABS thsn/cmm	NRBC #/100 WBC	SEG NEU thsn/cmm	BAND NEU thsn/cmm	LYMPH thsn/cmm	MONO thsn/cmm	EOSIN thsn/cmm	BASO thsn/cmm
1235	1 (VCTL)	0	97.2	0.0	5.0	0.1	3.0	0.3	0.1	0.0
1245	1 (VCTL)	0	75.6	0.0	6.7	0.2	2.8	0.4	0.0	0.0
1249	1 (VCTL)	0	57.2	0.0	7.4	0.0	2.0	0.3	0.0	0.0
1242	2	10	68.7	0.0	3.5	0.0	2.5	0.1	0.3	0.0
1246	2	10	44.9	0.0	5.7	0.7	1.4	1.0	0.2	0.0
1250	2	10	55.4	0.0	3.7	0.0	3.1	0.3	0.2	0.0
1238	3	30	72.6	0.0	4.8	0.0	1.0	0.4	0.0	0.0
1239	3	30	17.2	0.0	8.0	0.0	1.6	0.3	0.0	0.0
1243	3	30	35.8	0.0	6.1	0.1	3.1	0.6	0.1	0.0
1237	4	90/45[a]	7.5	0.0	11.1	0.0	1.8	1.0	0.1	0.0
1240	4	90/45	108.8	0.0	4.4	0.3	2.9	1.3	0.0	0.0
1241	4	90/45	31.0	0.0	5.6	0.7	3.1	0.7	0.0	0.0
1247	4	90/45	Dead	--	--	--	--	--	--	--
1248	4	90/45	Dead	--	--	--	--	--	--	--
1235	1 (VCTL)	0	70.5	0.0	8.0	0.6	4.7	1.7	0.2	0.0
1245	1 (VCTL)	0	149.5	1.0	7.8	0.3	4.9	0.7	1.0	0.0
1249	1 (VCTL)	0	117.2	0.0	9.4	0.0	4.3	0.7	0.3	0.0
1236	5	5	59.5	0.0	9.6	0.0	3.8	0.4	0.3	0.0
1244	5	5	86.7	0.0	7.7	0.5	2.3	1.1	0.6	0.0

[a] dose decreased from 90 to 45 µg/kg on Day 8

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-6 (cont.)

Individual Animal Hematology Data - Females

Post-dose

Animal Number	Group	Dose (µg/kg)	NRBC #/100 WBC	SEG NEU %	BAND NEU %	LYMPH %	MONO %	EOSIN %	BASO %
1235	1 (VCTL)	0	0	59	1	35	4	1	0
1245	1 (VCTL)	0	0	66	2	28	4	0	0
1249	1 (VCTL)	0	0	76	0	21	3	0	0
1242	2	10	0	55	0	39	2	4	0
1246	2	10	0	63	8	16	11	2	0
1250	2	10	0	50	0	43	4	3	0
1238	3	30	0	78	0	16	6	0	0
1239	3	30	0	81	0	16	3	0	0
1243	3	30	0	61	1	31	6	1	0
1237	4	90/45[a]	0	79	0	13	7	1	0
1240	4	90/45	0	49	3	33	15	0	0
1241	4	90/45	0	55	7	31	7	0	0
1247	4	90/45	Dead	--	--	--	--	--	--
1248	4	90/45	Dead	--	--	--	--	--	--
1235	1 (VCTL)	0	0	53	4	31	11	2	0
1245	1 (VCTL)	0	1	53	2	33	5	7	0
1249	1 (VCTL)	0	0	64	0	29	5	2	0
1236	5	5	0	68	0	27	3	2	0
1244	5	5	0	63	4	19	9	5	0

[a] dose decreased from 90 to 45 µg/kg on Day 8

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-7

Individual Animal Red Blood Cell Morphology Observations - Males
Pre-test

Animal Number	Group	Dose (µg/kg)	Observation
1252	1 (VCTL)	0	Anisocytosis +; Platelets Adequate and Normal
1256	1 (VCTL)	0	Polychromasia +; Anisocytosis +; Platelets Adequate and Normal
1258	1 (VCTL)	0	Anisocytosis +; Platelets Adequate and Normal
1263	1 (VCTL)	0	Polychromasia +; Anisocytosis +; Platelets Adequate and Normal
1266	1 (VCTL)	0	Polychromasia +; Anisocytosis +; Platelets Adequate and Normal
1257	2	10	Anisocytosis +; Platelets Adequate and Normal
1260	2	10	Anisocytosis +; Platelets Adequate and Normal
1262	2	10	Polychromasia +; Anisocytosis +; Microcytosis+, Platelets Adequate and Normal

Appendix C Table C-7 (cont.)

Individual Animal Red Blood Cell Morphology Observations - Males
Pre-test

Animal Number	Group	Dose (µg/kg)	Observation
1259	3	30	Polychromasia +; Anisocytosis +; Platelets Adequate and Normal
1261	3	30	Polychromasia +; Anisocytosis +; Microcytosis+, Platelets Adequate and Normal
1265	3	30	Polychromasia +; Anisocytosis +; Platelets Adequate and Normal
1251	4	90	Anisocytosis +; Platelets Adequate and Normal
1253	4	90	Polychromasia +; Anisocytosis +; Platelets Adequate and Normal
1254	4	90	Polychromasia +; Anisocytosis +; Howell-Jolly Bodies, Platelets Adequate and Normal
1255	4	90	Anisocytosis +; Platelets Adequate and Normal
1264	4	90	Anisocytosis +; Platelets Adequate and Normal

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-7 (cont.)

Individual Animal Red Blood Cell Morphology Observations - Females
Pre-test

Animal Number	Group	Dose (µg/kg)	Observation
1235	1 (VCTL)	0	Polychromasia +; Anisocytosis +; Platelets Adequate and Normal
1236	1 (VCTL)	0	Polychromasia +; Anisocytosis +; Platelets Adequate and Normal
1244	1 (VCTL)	0	Anisocytosis +; Platelets Adequate and Normal
1245	1 (VCTL)	0	Polychromasia +; Anisocytosis +; Platelets Adequate and Normal
1249	1 (VCTL)	0	Anisocytosis +; Microcytosis +; Platelets Adequate and Normal
1242	2	10	Polychromasia +; Anisocytosis +; Platelets Adequate and Normal
1246	2	10	Polychromasia +; Anisocytosis +; Howell-Jolly Bodies, Platelets Adequate and Normal
1250	2	10	Polychromasia +; Anisocytosis +; Platelets Adequate and Normal

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-7 (cont.)

Individual Animal Red Blood Cell Morphology Observations - Females
Pre-test

Animal Number	Group	Dose (µg/kg)	Observation
1238	3	30	Anisocytosis +; Platelets Adequate and Normal
1239	3	30	Anisocytosis +; Platelets Adequate and Normal
1243	3	30	Polychromasia +; Anisocytosis +; Platelets Adequate and Normal
1237	4	90	Anisocytosis +; Platelets Adequate and Normal
1240	4	90	Anisocytosis +; Platelets Adequate and Normal
1241	4	90	Anisocytosis +; Platelets Adequate and Normal
1247	4	90	Anisocytosis +; Platelets Adequate and Normal
1248	4	90	Polychromasia +; Anisocytosis +; Platelets Adequate and Normal

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-8

Individual Animal Red Blood Cell Morphology Observations - Males
Post-dose

Animal Number	Group	Dose (μg/kg)	Observation
1252	1 (VCTL)	0	Anisocytosis +; Platelets Adequate and Normal
1256	1 (VCTL)	0	Anisocytosis +; Platelets Adequate and Normal
1263	1 (VCTL)	0	Anisocytosis +; Platelets Adequate and Normal
1257	2	10	Anisocytosis +; Platelets Adequate and Normal
1260	2	10	Anisocytosis +; Platelets Adequate and Normal
1262	2	10	Anisocytosis +; Platelets Adequate and Normal
1259	3	30	Dead
1261	3	30	Polychromasia +; Anisocytosis +; Howell-Jolly Bodies; Platelets Adequate and Normal
1265	3	30	Anisocytosis +; Platelets Adequate and Normal
1251	4	90/45[a]	Anisocytosis +; Platelets Adequate and Normal
1253	4	90/45	Anisocytosis +; Poikilocytosis +; Platelets Decreased and Normal
1254	4	90/45	Anisocytosis +; Platelets Adequate and Normal
1255	4	90/45	Dead
1264	4	90/45	Anisocytosis +; Platelets Adequate and Normal

[a] dose decreased from 90 to 45 μg/kg on Day 9

Appendix C Table C-8 (cont.)

Individual Animal Red Blood Cell Morphology Observations - Females
Post-dose

Animal Number	Group	Dose (µg/kg)	Observation
1235	1 (VCTL)	0	Anisocytosis +; Platelets Adequate and Normal
1245	1 (VCTL)	0	Polychromasia +; Anisocytosis +; Platelets Adequate and Normal
1249	1 (VCTL)	0	Anisocytosis +; Microcytosis +; Platelets Adequate and Normal
1242	2	10	Anisocytosis +; Platelets Adequate and Normal
1246	2	10	Anisocytosis +; Poikilocytosis +; Platelets Adequate and Normal
1250	2	10	Anisocytosis +; Platelets Adequate and Normal
1238	3	30	Anisocytosis +; Platelets Adequate and Normal
1239	3	30	Anisocytosis +; Platelets Adequate and Normal
1243	3	30	Anisocytosis +; Platelets Adequate and Normal
1237	4	90/45[a]	Anisocytosis +; Platelets Adequate and Normal
1240	4	90/45	Anisocytosis +; Poikilocytosis +; Platelets Adequate and Normal
1241	4	90/45	Anisocytosis +; Poikilocytosis +; Platelets Adequate and Normal
1247	4	90/45	Dead
1248	4	90/45	Dead

[a] dose decreased from 90 to 45 µg/kg on Day 8

Appendix C Table C-8 (cont.)

Individual Animal Red Blood Cell Morphology Observations - Males

Post-dose

Animal Number	Group	Dose (µg/kg)	Observation
1252	1 (VCTL)	0	Polychromasia +; Anisocytosis+; Platelets Adequate and Normal
1256	1 (VCTL)	0	Anisocytosis+; Platelets Adequate and Normal
1263	1 (VCTL)	0	Polychromasia +; Anisocytosis+; Platelets Adequate and Normal
1258	5	5	Anisocytosis+; Platelets Decreased and Normal
1266	5	5	Polychromasia +; Anisocytosis+; Platelets Adequate and Normal

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-8 (cont.)

Individual Animal Red Blood Cell Morphology Observations - Females

Post-dose

Animal Number	Group	Dose (μg/kg)	Observation
1235	1 (VCTL)	0	Anisocytosis+; Platelets Adequate and Normal
1245	1 (VCTL)	0	Polychromasia +; Anisocytosis+; Howell-Jolly Bodies; Platelets Adequate and Normal
1249	1 (VCTL)	0	Polychromasia +; Anisocytosis+; Target Cells +; Platelets Adequate and Normal
1236	5	5	Anisocytosis+; Platelets Adequate and Normal
1244	5	5	Polychromasia +; Anisocytosis+; Platelets Adequate and Normal

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-9

Individual Animal Coagulation Data - Males

Pre-test

Animal Number	Group	Dose (µg/kg)	PT sec	APTT sec	FIB mg/dL
1252	1 (VCTL)	0	8.7	11.8	170
1256	1 (VCTL)	0	8.7	10.4	165
1258	1 (VCTL)	0	8.5	11.9	246
1263	1 (VCTL)	0	8.8	10.6	164
1266	1 (VCTL)	0	8.8	11.0	178
1257	2	10	8.7	9.8	230
1260	2	10	8.6	12.5	187
1262	2	10	8.5	12.4	191
1259	3	30	8.5	9.9	214
1261	3	30	8.4	9.9	212
1265	3	30	8.7	10.6	165
1251	4	90	8.8	10.4	258
1253	4	90	8.6	10.1	184
1254	4	90	16.2	10.5	185
1255	4	90	8.9	11.5	210
1264	4	90	8.3	11.9	201

Appendix C Table C-9 (cont.)

Individual Animal Coagulation Data - Females

Pre-test

Animal Number	Group	Dose (μg/kg)	PT sec	APTT sec	FIB mg/dL
1235	1 (VCTL)	0	8.7	9.7	167
1236	1 (VCTL)	0	9.1	11.3	146
1244	1 (VCTL)	0	8.7	11.2	157
1245	1 (VCTL)	0	9.1	10.9	145
1249	1 (VCTL)	0	8.7	10.8	188
1242	2	10	8.6	11.5	243
1246	2	10	9.0	10.8	138
1250	2	10	8.7	12.3	153
1238	3	30	8.8	12.0	132
1239	3	30	8.7	10.2	151
1243	3	30	8.7	10.9	155
1237	4	90	8.6	11.7	182
1240	4	90	8.6	11.7	177
1241	4	90	8.6	11.1	243
1247	4	90	9.2	10.3	178
1248	4	90	8.9	12.5	172

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-10

Individual Animal Coagulation Data - Males

Post-dose

Animal Number	Group	Dose (µg/kg)	PT sec	APTT sec	FIB mg/dL
1252	1 (VCTL)	0	7.7	10.0	174
1256	1 (VCTL)	0	7.9	10.4	172
1263	1 (VCTL)	0	7.6	10.0	194
1257	2	10	7.2	12.5	498
1260	2	10	7.5	11.8	300
1262	2	10	7.6	14.3	243
1259	3	30	Dead	--	--
1261	3	30	7.4	14.9	560
1265	3	30	7.4	13.2	272
1251	4	90/45[a]	8.2	17.9	291
1253	4	90/45	14.4	106.0	309
1254	4	90/45	12.1	12.4	258
1255	4	90/45	Dead	--	--
1264	4	90/45	7.2	14.0	408
1252	1 (VCTL)	0	7.7	9.8	236
1256	1 (VCTL)	0	8.1	10.6	152
1263	1 (VCTL)	0	7.7	10.5	159
1258	5	5	8.0	11.4	268
1266	5	5	7.9	10.4	281

[a] dose decreased from 90 to 45 µg/kg on Day 9

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-10 (cont.)

Individual Animal Coagulation Data - Females

Post-dose

Animal Number	Group	Dose (µg/kg)	PT sec	APTT sec	FIB mg/dL
1235	1 (VCTL)	0	7.7	9.6	159
1245	1 (VCTL)	0	7.5	10.2	188
1249	1 (VCTL)	0	7.8	9.7	164
1242	2	10	7.6	12.5	205
1246	2	10	7.5	10.3	265
1250	2	10	7.5	11.8	243
1238	3	30	7.7	15.5	208
1239	3	30	7.9	14.5	154
1243	3	30	7.3	13.5	233
1237	4	90/45[a]	7.4	14.2	233
1240	4	90/45	7.4	13.3	319
1241	4	90/45	7.2	13.4	309
1247	4	90/45	Dead	--	--
1248	4	90/45	Dead	--	--
1235	1 (VCTL)	0	7.7	10.1	145
1245	1 (VCTL)	0	7.7	9.8	159
1249	1 (VCTL)	0	7.7	10.5	160
1236	5	5	8.0	10.4	186
1244	5	5	7.7	11.1	178

[a] dose decreased from 90 to 45 µg/kg on Day 8

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-11

Individual Animal Clinical Chemistry Data - Males

Pre-test

Animal Number	Group	Dose (µg/kg)	NA mmol/L	K mmol/L	CL mmol/L	CA mg/dL	PO4 mg/dL	ALP IU/L	ALT IU/L	AST IU/L	GGT IU/L	LDH IU/L
1252	1 (VCTL)	0	145	4.6	111	11.1	7.1	130	25	29	3	200
1256	1 (VCTL)	0	146	4.7	110	11.1	7.3	105	28	27	4	183
1258	1 (VCTL)	0	148	4.7	114	10.9	7.4	91	29	38	2	145
1263	1 (VCTL)	0	145	4.6	111	11.4	7.9	119	30	36	2	130
1266	1 (VCTL)	0	146	5.4	110	12.0	8.3	111	19	29	3	231
1257	2	10	146	4.9	112	10.7	6.4	148	41	30	4	115
1260	2	10	144	4.6	111	11.0	7.6	113	32	27	1	106
1262	2	10	146	4.9	112	11.1	8.1	108	50	33	3	132
1259	3	30	147	5.5	106	11.9	8.6	89	32	34	4	97
1261	3	30	147	4.6	113	11.4	6.7	96	35	34	4	119
1265	3	30	145	4.7	111	11.7	8.3	103	22	33	3	229
1251	4	90	146	5.7	108	12.0	8.3	122	23	36	4	240
1253	4	90	145	5.3	109	10.5	7.0	116	47	28	4	104
1254	4	90	144	5.1	111	11.5	7.6	74	33	29	2	158
1255	4	90	145	5.1	112	11.2	7.3	104	32	26	4	114
1264	4	90	146	4.3	110	11.3	7.1	154	29	46	1	378

Appendix C Table C-11 (cont.)

Individual Animal Clinical Chemistry Data - Males

Pre-test

Animal Number	Group	Dose (µg/kg)	TBIL mg/dL	BUN mg/dL	CREA mg/dL	GLU mg/dL	TP g/dL	ALB g/dL	GLOB g/dL	A/G -	CHOL mg/dL	TG mg/dL
1252	1 (VCTL)	0	0.28	10	0.9	104	5.4	3.1	2.3	1.3	157	14
1256	1 (VCTL)	0	0.44	11	0.7	107	5.5	3.3	2.2	1.5	158	16
1258	1 (VCTL)	0	0.37	11	0.8	98	5.3	3.1	2.2	1.4	171	29
1263	1(VCTL)	0	0.36	13	0.6	88	5.1	3.3	1.8	1.8	117	24
1266	1 (VCTL)	0	0.40	12	0.6	110	5.6	3.3	2.3	1.4	148	20
1257	2	10	0.51	12	0.7	106	5.2	3.0	2.2	1.4	121	30
1260	2	10	0.50	15	0.9	96	5.1	3.1	2.0	1.6	169	27
1262	2	10	0.27	9	0.7	76	4.9	3.1	1.8	1.7	108	19
1259	3	30	0.34	11	0.8	113	5.9	3.3	2.6	1.3	150	17
1261	3	30	0.16	13	0.8	96	5.3	3.4	1.9	1.8	138	23
1265	3	30	0.31	18	0.6	101	5.4	3.4	2.0	1.7	144	32
1251	4	90	0.25	14	0.9	109	6.1	3.3	2.8	1.2	167	38
1253	4	90	0.18	11	0.6	105	5.5	3.2	2.3	1.4	150	21
1254	4	90	0.50	10	0.5	93	5.2	3.2	2.0	1.6	134	14
1255	4	90	0.35	10	0.7	90	5.1	3.0	2.1	1.4	131	18
1264	4	90	0.43	15	0.7	113	5.5	3.6	1.9	1.9	139	17

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-11 (cont.)

Individual Animal Clinical Chemistry Data - Females

Pre-test

Animal Number	Group	Dose (µg/kg)	NA mmol/L	K mmol/L	CL mmol/L	CA mg/dL	PO4 mg/dL	ALP IU/L	ALT IU/L	AST IU/L	GGT IU/L	LDH IU/L
1235	1 (VCTL)	0	146	5.0	110	11.4	6.6	125	32	32	6	195
1236	1 (VCTL)	0	146	4.5	110	11.3	6.4	82	36	30	4	149
1244	1 (VCTL)	0	148	4.8	113	11.5	7.7	96	31	34	3	97
1245	1 (VCTL)	0	148	5.0	111	11.1	6.5	109	36	30	3	227
1249	1 (VCTL)	0	144	4.9	109	11.2	7.4	106	49	35	2	348
1242	2	10	147	4.8	111	11.2	7.3	72	25	30	3	223
1246	2	10	146	4.6	112	11.3	5.9	104	36	24	3	90
1250	2	10	146	4.5	109	11.4	7.4	103	22	38	3	388
1238	3	30	147	5.4	109	11.2	6.9	120	39	26	4	125
1239	3	30	149	5.1	111	11.1	7.3	117	43	24	3	154
1243	3	30	145	5.1	109	11.2	6.7	79	36	35	5	243
1237	4	90	147	5.0	110	11.4	6.2	111	29	30	4	235
1240	4	90	145	4.8	108	11.3	6.7	81	31	33	4	192
1241	4	90	147	4.2	112	10.9	6.5	70	30	30	5	129
1247	4	90	145	5.0	111	11.4	7.0	75	35	31	4	92
1248	4	90	147	5.1	111	11.4	8.0	137	29	43	4	511

Appendix C Table C-11 (cont.)

Individual Animal Clinical Chemistry Data - Females

Pre-test

Animal Number	Group	Dose (µg/kg)	TBIL mg/dL	BUN mg/dL	CREA mg/dL	GLU mg/dL	TP g/dL	ALB g/dL	GLOB g/dL	A/G -	CHOL mg/dL	TG mg/dL
1235	1 (VCTL)	0	0.37	10	0.8	91	5.7	3.5	2.2	1.6	134	20
1236	1 (VCTL)	0	0.53	12	0.6	102	5.4	3.4	2.0	1.7	116	15
1244	1 (VCTL)	0	0.33	11	0.7	96	5.2	3.2	2.0	1.6	185	23
1245	1 (VCTL)	0	0.35	9	0.7	93	5.1	3.2	1.9	1.7	114	24
1249	1 (VCTL)	0	0.43	12	0.7	92	5.1	3.2	1.9	1.7	157	25
1242	2	10	0.40	14	0.8	94	5.3	3.2	2.1	1.5	143	17
1246	2	10	0.46	12	0.8	98	5.4	3.4	2.0	1.7	136	18
1250	2	10	0.37	11	0.9	97	5.2	3.3	1.9	1.7	133	22
1238	3	30	0.27	9	0.7	94	5.5	3.4	2.1	1.6	163	30
1239	3	30	0.33	15	0.7	88	5.3	3.5	1.8	1.9	137	17
1243	3	30	0.28	12	0.7	96	5.2	3.1	2.1	1.5	115	20
1237	4	90	0.30	11	0.6	89	5.6	3.3	2.3	1.4	149	21
1240	4	90	0.34	13	0.8	88	5.3	3.3	2.0	1.7	142	28
1241	4	90	0.42	12	0.6	89	5.4	3.1	2.3	1.3	152	22
1247	4	90	0.25	15	0.8	97	5.2	3.0	2.2	1.4	121	19
1248	4	90	0.77	12	0.6	105	5.5	3.5	2.0	1.8	167	16

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-12

Individual Animal Clinical Chemistry Data - Males

Post-dose

Animal Number	Group	Dose (µg/kg)	NA mmol/L	K mmol/L	CL mmol/L	CA mg/dL	PO4 mg/dL	ALP IU/L	ALT IU/L	AST IU/L	GGT IU/L	LDH IU/L
1252	1 (VCTL)	0	147	4.9	108	11.2	6.4	128	23	35	4	186
1256	1 (VCTL)	0	145	4.7	108	10.8	6.9	100	29	30	6	169
1263	1 (VCTL)	0	145	4.5	110	11.4	7.9	110	25	37	5	134
1257	2	10	141	5.2	101	15.0	5.1	52	24	30	6	280
1260	2	10	144	4.6	106	14.9	5.6	63	44	30	4	137
1262	2	10	144	4.2	105	14.8	5.9	31	33	23	4	245
1259	3	30	Dead	--	--	--	--	--	--	--	--	--
1261	3	30	153	5.0	110	15.3	5.7	42	16	42	5	289
1265	3	30	141	4.4	105	18.6	5.0	80	27	38	6	510
1251	4	90/45[a]	147	4.2	112	14.1	6.2	55	32	51	7	509
1253	4	90/45	152	3.8	114	17.2	5.3	134	86	80	5	166
1254	4	90/45	146	4.1	114	17.4	4.9	37	48	43	7	215
1255	4	90/45	Dead	--	--	--	--	--	--	--	--	--
1264	4	90/45	142	4.5	107	16.8	4.7	71	18	29	4	336
1252	1 (VCTL)	0	144	4.6	110	11.0	6.6	112	29	32	5	160
1256	1 (VCTL)	0	143	5.1	109	11.0	6.7	93	36	33	5	133
1263	1 (VCTL)	0	143	4.3	109	11.4	7.1	106	26	44	1	138
1258	5	5	143	5.2	105	11.9	6.7	78	34	38	3	101
1266	5	5	142	4.4	104	13.6	4.7	96	28	40	4	161

[a] dose decreased from 90 to 45 µg/kg on Day 9

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-12 (cont.)

Individual Animal Clinical Chemistry Data - Males

Post-dose

Animal Number	Group	Dose (µg/kg)	TBIL mg/dL	BUN mg/dL	CREA mg/dL	GLU mg/dL	TP g/dL	ALB g/dL	GLOB g/dL	A/G -	CHOL mg/dL	TG mg/dL
1252	1 (VCTL)	0	0.66	12	0.7	87	5.2	3.1	2.1	1.5	135	QNS[a]
1256	1 (VCTL)	0	0.31	12	0.7	92	5.5	3.2	2.3	1.4	142	28
1263	1(VCTL)	0	0.45	11	0.8	83	5.1	3.1	2.0	1.6	116	18
1257	2	10	0.30	28	1.1	81	5.6	3.0	2.6	1.2	220	47
1260	2	10	0.53	36	0.9	82	5.2	3.0	2.2	1.4	177	26
1262	2	10	0.34	40	0.9	82	5.6	3.4	2.2	1.5	140	32
1259	3	30	Dead	--	--	--	--	--	--	--	--	--
1261	3	30	0.35	40	0.6	81	6.3	3.1	3.2	1.0	234	76
1265	3	30	0.22	38	1.3	110	5.3	3.2	2.1	1.5	190	45
1251	4	90/45[b]	0.43	74	0.6	84	5.4	2.9	2.5	1.2	147	14
1253	4	90/45	0.51	61	0.4	5	4.2	2.3	1.9	1.2	183	30
1254	4	90/45	0.54	40	0.6	99	4.8	2.6	2.2	1.2	148	27
1255	4	90/45	Dead	--	--	--	--	--	--	--	--	--
1264	4	90/45	0.19	27	0.8	112	5.0	3.0	2.0	1.5	164	35
1252	1 (VCTL)	0	0.61	14	0.8	92	5.5	3.1	2.4	1.3	158	36
1256	1 (VCTL)	0	0.30	15	0.8	105	5.7	3.3	2.4	1.4	144	23
1263	1 (VCTL)	0	0.44	17	0.7	94	5.4	3.2	2.2	1.5	126	19
1258	5	5	0.40	16	0.8	112	5.4	3.0	2.4	1.3	165	31
1266	5	5	0.41	16	0.8	98	5.9	3.3	2.6	1.3	151	39

[a] QNS = quantity not sufficient
[b] dose decreased from 90 to 45 µg/kg on Day 9

Appendix C Table C-12 (cont.)

Individual Animal Clinical Chemistry Data - Females

Post-dose

Animal Number	Group	Dose (μg/kg)	NA mmol/L	K mmol/L	CL mmol/L	CA mg/dL	PO4 mg/dL	ALP IU/L	ALT IU/L	AST IU/L	GGT IU/L	LDH IU/L
1235	1 (VCTL)	0	144	4.7	110	11.3	6.3	117	33	31	3	137
1245	1 (VCTL)	0	145	4.8	110	10.9	7.2	117	29	36	5	161
1249	1 (VCTL	0	143	4.5	110	11.2	6.5	99	38	30	5	196
1242	2	10	144	4.6	107	14.8	5.6	58	31	28	6	230
1246	2	10	143	4.4	110	13.8	4.9	73	33	21	5	84
1250	2	10	144	4.3	107	15.4	5.7	67	19	28	4	396
1238	3	30	144	4.6	103	15.6	5.4	66	24	24	8	298
1239	3	30	143	4.7	107	13.8	5.4	39	34	25	6	465
1243	3	30	144	4.8	107	16.4	5.5	33	28	37	4	580
1237	4	90/45[a]	147	4.6	110	18.0	4.8	88	33	38	6	279
1240	4	90/45	141	3.7	101	17.4	4.9	60	34	22	6	69
1241	4	90/45	141	4.5	107	17.2	4.7	61	34	37	4	145
1247	4	90/45	Dead	--	--	--	--	--	--	--	--	--
1248	4	90/45	Dead	--	--	--	--	--	--	--	--	--
1235	1 (VCTL)	0	143	4.8	112	10.8	6.9	123	36	31	3	121
1245	1 (VCTL)	0	143	5.1	110	10.8	6.9	119	34	42	5	174
1249	1 (VCTL)	0	143	4.7	107	11.5	6.5	93	41	43	3	252
1236	5	5	145	5.4	105	13.0	5.6	83	46	37	5	126
1244	5	5	142	4.4	109	12.0	6.0	74	40	53	3	139

[a] dose decreased from 90 to 45 μg/kg on Day 8

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-12 (cont.)

Individual Animal Clinical Chemistry Data - Females

Post-dose

Animal Number	Group	Dose (μg/kg)	TBIL mg/dL	BUN mg/dL	CREA mg/dL	GLU mg/dL	TP g/dL	ALB g/dL	GLOB g/dL	A/G -	CHOL mg/dL	TG mg/dL
1235	1 (VCTL)	0	0.40	9	0.7	95	5.6	3.5	2.1	1.7	122	19
1245	1 (VCTL)	0	0.33	12	0.8	84	5.2	3.1	2.1	1.5	99	17
1249	1 (VCTL)	0	0.37	14	0.9	96	5.2	3.3	1.9	1.7	199	16
1242	2	10	0.49	25	0.8	84	5.6	3.3	2.3	1.4	168	25
1246	2	10	0.39	20	0.8	90	5.4	3.3	2.1	1.6	162	22
1250	2	10	0.57	21	0.9	99	5.7	3.5	2.2	1.6	193	25
1238	3	30	0.50	24	0.8	85	5.8	3.3	2.5	1.3	232	42
1239	3	30	0.54	42	0.5	101	4.8	3.1	1.7	1.8	139	28
1243	3	30	0.52	39	0.8	105	4.7	2.8	1.9	1.5	180	57
1237	4	90/45[a]	0.40	28	0.7	93	5.2	3.0	2.2	1.4	217	36
1240	4	90/45	0.42	27	1.1	91	5.3	3.0	2.3	1.3	318	76
1241	4	90/45	0.55	37	1.0	94	5.5	3.1	2.4	1.3	246	47
1247	4	90/45	Dead	--	--	--	--	--	--	--	--	--
1248	4	90/45	Dead	--	--	--	--	--	--	--	--	--
1235	1 (VCTL)	0	0.45	15	0.7	101	5.6	3.5	2.1	1.7	117	27
1245	1 (VCTL)	0	0.38	15	0.9	93	5.3	3.0	2.3	1.3	104	31
1249	1 (VCTL)	0	0.46	19	1.1	101	5.4	3.3	2.1	1.6	198	25
1236	5	5	0.46	18	0.9	108	5.7	3.5	2.2	1.6	125	34
1244	5	5	0.53	17	0.8	108	5.2	3.1	2.1	1.5	156	32

[a] dose decreased from 90 to 45 μg/kg on Day 8

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-13

Urinalysis Key

SCALE (Microscopic Analysis):

1 =Occasional noted
2 =Noted in every field
3 =Large amounts in every field
4 =Full fields

HPF =High Power Field (400x) or (40x10)
LPF =Low Power Field (100x) or (10x10)
Casts =number/LPF
EP Cells =number/HPF
P04 =Scale
MR/NMR =Scale
WBC =number/HPF
RBC =number/HPF
Other =Scale
Sperm =Scale

CODE(Microscopic):

MR =Motile Rods
NMR =Nonmotile Rods
P04 =Phosphate Crystals, Triple

EP =Epithelial
RBC =Red Blood Cell(s)
WBC =White Blood Cell(s)
F =Fine
GRAN =Granular
+ =Positive
- =Negative

ABBREVIATIONS:

RI =Refractive Index
SG =Specific Gravity
LEU =Leucocytes
NIT =Nitrite
PRO =Protein
GLU =Glucose
KET =Ketones
UBG =Urobilinogen
BIL =Bilirubin
BLD =Blood
TR =Trace
Neg or 0 =Negative
Norm =Normal

LEU	PRO	GLU	KET	UBG
0 =Neg	0 =Neg	0 =Neg	0 =Neg	1 =1mg/dl
Tr =25/ul	Tr =15mg/dl	Tr =50mg/dl	Tr =5mg/dl	2 =4mg/dl
1 =100/ul	1 =30mg/dl	1 =100mg/dl	1 =15mg/dl	3 =8mg/dl
2 =250/ul	2 =100mg/dl	2 =250mg/dl	2 =50mg/dl	4 =12mg/dl
	3 =500mg/dl	4 =1000mg/dl	3 =150mg/dl	

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-14

Individual Animal Urinalysis Data - Males

Pre-test

Group (Dose) (µg/kg)	Animal Number	Color	Clarity	Volume	RI	SG	PH	LEU	NIT	PRO	GLU	KET	UBG	BIL	BLD	Casts	EP cells	P04	NMR	MR	RBC	WBC	Other
1 (VCTL; 0)	1252	Yellow	Cloudy	50	1.3482	1.035	6.5	2	+	0	0	0	Normal	0	3	0	3-6	1	4	4	4-8	6-10	0
	1256	Yellow	Cloudy	18	1.3520	1.045	6.5	2	+	1	0	0	Normal	0	2	0	6-10	3	4	4	4-8	6-10	Feces+
	1258	Yellow	Cloudy	62	1.3432	1.023	6.0	2	+	Trace	0	0	Normal	0	3	0	6-10	1	4	4	4-8	8-12	0
	1263	Yellow	Cloudy	26	1.3498	1.040	7.0	Trace	0	1	0	0	Normal	0	1	0	3-6	2	3	3	0-2	0-3	0
	1266	Yellow	Cloudy	44	1.3528	1.047	6.5	2	+	1	0	0	Normal	0	Trace	0	6-10	3	4	4	1-3	6-10	Feces+
2 (10)	1257	Yellow	Sl.Cloudy	90	1.3442	1.026	8.0	2	+	1	0	0	Normal	0	1	0	1-3	1	4	4	4-8	6-10	0
	1260	Yellow	Cloudy	10	1.3548	1.052	6.5	2	+	1	0	0	1	1	3	0	6-10	3	4	4	4-8	8-12	Feces+
	1262	Yellow	Cloudy	106	1.3436	1.026	9.0	2	0	0	0	0	Normal	0	1	0	6-10	2	4	4	6-10	8-12	Feces+
3 (30)	1259	Yellow	Sl.Cloudy	22	1.3464	1.030	6.0	2	+	Trace	0	0	Normal	0	1	0	6-10	1	4	4	4-8	6-10	0
	1261	Yellow	Cloudy	88	1.3458	1.030	7.0	Trace	0	1	0	0	Normal	0	4	0	3-6	3	4	4	1-3	3-5	Feces+
	1265	Yellow	Cloudy	65	1.3510	1.042	6.5	2	+	1	0	0	Normal	1	1	0	6-10	3	4	4	4-8	6-10	Feces+
4 (90)	1251	Yellow	Cloudy	80	1.3400	1.016	7.0	1	0	Trace	0	0	Normal	0	2	0	1-3	0	4	4	1-3	3-5	0
	1253	Yellow	Cloudy	22	1.3532	1.047	7.0	1	+	1	0	0	Normal	1	3	0	6-10	4	4	4	1-3	8-12	0
	1254	Yellow	Cloudy	31	1.3432	1.023	6.0	Trace	0	1	0	0	Normal	0	2	0	3-6	0	4	4	0-2	0-3	0
	1255	Yellow	Clear	17	1.3398	1.016	5.0	0	0	0	0	0	Normal	0	0	0	1-3	0	1	1	0-2	0-3	0
	1264	Yellow	Cloudy	85	1.3428	1.023	8.0	1	0	1	0	0	Normal	0	2	0	1-3	1	4	4	0-2	0-3	0

Urinalysis Parameters - Observations and Measurements - Male Dogs

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-14 (cont.)

Individual Animal Urinalysis Data - Females

Pre-test

Group (Dose)	Animal	Urinalysis Parameters - Observations and Measurements - Male Dogs																					
(μg/kg)	Number	Color	Clarity	Volume	RI	SG	PH	LEU	NIT	PRO	GLU	KET	UBG	BIL	BLD	Casts	EP cells	P04	NMR	MR	RBC	WBC	Other
1 (VCTL; 0)	1235	Yellow	Cloudy	59	1.3428	1.023	6.5	2	0	1	0	0	Normal	0	2	0	6-10	0	4	4	0-2	3-5	0
	1236	Yellow	Cloudy	35	1.3498	1.040	6.5	1	0	1	0	0	Normal	0	1	0	6-10	0	4	4	0-2	0-3	0
	1244	Yellow	Cloudy	45	1.3418	1.021	9.0	2	0	1	0	0	Normal	0	3	0	6-10	4	4	4	6-10	8-12	Feces+
	1245	Yellow	Cloudy	19	1.3430	1.023	6.0	2	+	Trace	0	0	Normal	0	3	0	6-10	0	4	4	4-8	6-10	0
	1249	Yellow	Cloudy	29	1.3432	1.023	6.5	2	0	1	0	0	Normal	0	3	0	6-10	0	4	4	6-10	6-10	0
2 (10)	1242	Yellow	Cloudy	52	1.3494	1.038	9.0	2	0	0	0	0	Normal	0	3	0	6-10	3	4	4	4-8	8-12	Feces+
	1246	Yellow	Sl.Cloudy	39	1.3446	1.028	7.0	2	+	Trace	0	0	Normal	0	2	0	6-10	1	3	4	0-2	3-5	0
	1250	Yellow	Cloudy	80	1.3512	1.042	8.0	2	0	1	0	0	Normal	0	4	0	6-10	3	4	4	6-10	8-12	Feces+
	1238	Yellow	Sl.Cloudy	39	1.3510	1.042	6.0	2	+	Trace	0	0	Normal	1	Trace	0	6-10	1	3	3	4-8	6-10	0
3 (30)	1239	Yellow	Sl.Cloudy	52	1.3508	1.042	6.0	0	0	Trace	0	0	Normal	1	0	0	3-6	0	2	2	0-2	3-5	0
	1243	Yellow	Cloudy	45	1.3418	1.021	7.0	2	0	Trace	Trace	0	Normal	0	3	0	6-10	3	4	4	6-10	8-12	Feces+
	1237	Yellow	Cloudy	56	1.3428	1.023	6.5	2	0	Trace	0	0	Normal	0	2	0	6-10	0	4	4	0-2	6-10	0
	1240	Yellow	Cloudy	40	1.3420	1.021	8.0	1	0	Trace	0	0	Normal	0	4	0	6-10	2	4	4	4-8	8-12	Feces+
4 (90)	1241	Yellow	Cloudy	24	1.3568	1.057	7.0	2	+	1	0	0	Normal	0	4	0	6-10	3	4	4	6-10	8-12	Feces+
	1247	Yellow	Cloudy	53	1.3490	1.038	6.5	2	+	1	0	0	Normal	1	2	0	6-10	1	4	4	4-8	8-12	Feces+
	1248	Yellow	Cloudy	67	1.3422	1.021	7.0	2	+	0	0	0	Normal	0	3	0	6-10	3	4	4	6-10	8-12	0

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-15

Individual Animal Urinalysis Data - Males

Post-dose

Group	Animal	Urinalysis Parameters - Observations and Measurements - Male Dogs																				
(µg/kg)	Number	Color	Clarity	Volume	RI	SG	PH	LEU	NIT	PRO	GLU	KET	UBG	BIL	BLD	Casts	EP cells	P04	NMR	MR	RBC	WBC	Other
1 (VCTL; 0)	1252	Yellow	Clear	7	1.3428	1.023	7.0	0	0	Trace	0	0	Normal	0	0	0	0	0	0	0	0	0	0
	1256	Yellow	Clear	4	1.3452	1.028	9.0	0	0	0	0	0	Normal	0	0	0	1-3	0	0	0	0	0-3	0
	1263	Yellow	Clear	19	1.3432	1.023	7.0	0	0	0	0	0	Normal	0	0	0	0	0	0	0	0-2	0	0
2 (10)																							
	1257	Pale Yellow	Clear	3	1.3368	1.009	5.0	0	0	Trace	0	0	Normal	0	0	0	0	0	0	0	0	0-3	0
	1260	Yellow	Clear	10	1.3382	1.012	6.0	0	0	Trace	0	0	Normal	0	Trace	0	1-3	0	0	0	1-3	0-3	0
	1262	Pale Yellow	Clear	5	1.3374	1.010	7.0	0	0	Trace	0	0	Normal	0	Trace	0	6-10	0	0	0	1-3	0-3	0
3 (30)	1259	Dead	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	1261	Dead	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	1265	Yellow	Clear	1	1.3498	1.040	6.5	Trace	0	2	0	Trace	Normal	1	2	0	6-10	0	0	0	6-10	3-5	0
4 (90/45[a])	1251	Dead	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	1253	Dead	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	1254	Yellow	Clear	8	1.3520	1.045	6.5	0	0	1	0	0	Normal	1	2	0	3-6	0	0	0	6-10	0	0
	1255	Dead	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	1264	Yellow	Clear	5	1.3474	1.033	6.5	0	0	1	0	Trace	Normal	1	0	0	1-3	0	0	0	1-3	0-3	0
5 (5)	1258	Yellow	Sl.Cloudy	10	1.3428	1.023	8.0	0	0	Trace	0	0	Normal	0	0	0	3-6	0	2	0	0	0-3	0
	1266	Pale Yellow	Clear	5	1.3350	1.005	8.0	0	0	0	0	0	Normal	0	1	0	3-6	0	2	0	4-8	0-3	0

[a] dose decreased from 90 to 45 µg/kg on Day 9

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-15 (cont.)

Individual Animal Urinalysis Data - Females

Post-dose

Group (Dose) (µg/kg)	Animal Number	Color	Clarity	Volume	RI	SG	PH	LEU	NIT	PRO	GLU	KET	UBG	BIL	BLD	Casts	EP cells	P04	NMR	MR	RBC	WBC	Other
		Urinalysis Parameters - Observations and Measurements - Female Dogs																					
1 (VCTL; 0)	1235	Yellow	Sl.Cloudy	7	1.3450	1.028	8.0	0	0	Trace	0	0	Normal	0	Trace	0	6-10	0	0	0	4-8	0-3	0
	1245	Yellow	Clear	7	1.3448	1.028	8.0	0	0	Trace	0	0	Normal	0	0	0	3-6	0	0	0	0	0	0
	1249	Yellow	Sl.Cloudy	7	1.3424	1.022	7.0	Trace	0	Trace	0	0	Normal	0	1	0	6-10	0	3	0	4-8	6-10	0
2 (10)																							
	1242	Yellow	Clear	4	1.3428	1.023	7.0	0	0	Trace	0	0	Normal	0	1	0	3-6	0	0	0	4-8	0-3	0
	1246	Yellow	Clear	5	1.3430	1.023	6.5	0	0	1	0	0	Normal	0	1	0	6-10	0	0	0	4-8	0-3	0
	1250	Yellow	Clear	5	1.3382	1.012	5.0	0	0	Trace	0	0	Normal	0	Trace	0	3-6	0	0	0	1-3	0-3	0
3 (30)	1238	Yellow	Clear	2	1.3490	1.038	5.0	0	0	1	0	Trace	Normal	0	1	0	20-30	0	0	0	1-3	0-3	0
	1239	Yellow	Clear	7	1.3438	1.026	5.0	0	0	Trace	0	0	Normal	0	3	0	3-6	0	0	0	30-5	3-5	0
	1243	Dead	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
4 (90/45[a])	1237	Yellow	Clear	10	1.3510	1.042	7.0	Trace	0	1	0	Trace	Normal	0	0	0	1-3	0	0	0	0-2	0-3	0
	1240	Pale Yellow	Clear	1	1.3382	1.012	5.0	0	0	2	0	0	Normal	0	2	0	20-30	0	0	0	4-8	0-3	0
	1241	Pale Yellow	Clear	1	1.3388	1.014	5.0	0	0	1	0	0	Normal	0	1	0	1-3	0	0	0	1-3	0-3	0
	1247	Dead	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	1248	Dead	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
5 (5)	1236	Pale Yellow	Sl.Cloudy	9	1.3358	1.007	8.0	0	0	0	0	0	Normal	0	1	0	6-10	0	0	0	4-8	0-3	0
	1244	Pale Yellow	Sl.Cloudy	5	1.3392	1.014	9.0	0	0	0	0	0	Normal	0	Trace	0	3-6	0	2	0	1-3	0-3	0

[a] dose decreased from 90 to 45 µg/kg on Day 8

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-16

Individual Animal Absolute Organ Weights (g) - Males

Animal Number	Dose	Adrenals	Brain	Heart	Kidney (Left)	Kidney (Right)	Liver	Spleen	Testes	Thymus	Thyroid[a]
1252	0	0.762	72.70	73.45	19.86	19.75	250.03	21.41	9.00	13.81	1.236
1256	0	0.797	68.17	75.64	20.90	20.58	264.41	19.21	9.07	17.86	1.097
1258	0	Moved[b]	--	--	--	--	--	--	--	--	--
1263	0	0.909	72.01	71.68	20.59	20.63	278.12	22.43	5.72	20.84	1.134
1266	0	Moved	--	--	--	--	--	--	--	--	--
1258	5	0.953	74.81	71.94	19.61	19.75	272.25	28.71	3.48	11.43	1.385
1266	5	0.940	75.34	85.22	23.74	23.35	247.44	24.64	9.25	15.32	1.809
1257	10	0.773	67.63	41.88	16.89	16.06	188.97	15.72	3.98	3.48	0.923
1260	10	0.941	72.13	47.72	19.46	18.56	186.81	12.52	1.89	2.63	0.840
1262	10	1.021	76.34	46.42	25.74	24.28	203.08	18.77	1.15	2.44	0.753
1259	30	Dead	--	--	--	--	--	--	--	--	--
1261	30	Dead	--	--	--	--	--	--	--	--	--
1265	30	0.959	69.18	45.75	16.45	15.46	149.52	14.57	1.72	3.12	1.195
1251	90/45[c]	Dead	--	--	--	--	--	--	--	--	--
1253	90/45	Dead	--	--	--	--	--	--	--	--	--
1254	90/45	1.001	73.16	44.07	22.65	21.26	151.23	9.90	2.62	2.07	0.749
1255	90/45	Dead	--	--	--	--	--	--	--	--	--
1264	90/45	0.857	64.76	41.53	15.30	14.80	163.51	9.99	1.82	2.04	0.956

[a] thyroids, including parathyroids
[b] switched to 5 μg/kg group (Group 5) on Day 8
[c] dose decreased from 90 to 45 μg/kg on Day 9

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-16 (cont.)

Individual Animal Absolute Organ Weights (g) - Females

Animal Number	Dose	Adrenals	Brain	Heart	Kidney (Left)	Kidney (Right)	Liver	Ovaries	Spleen	Thymus	Thyroid[a]
1235	0	0.854	72.98	64.19	17.80	19.74	244.18	1.376	19.41	11.25	0.904
1236	0	Moved[b]	--	--	--	--	--	--	--	--	--
1244	0	Moved	--	--	--	--	--	--	--	--	--
1245	0	0.770	68.69	65.78	18.59	18.60	233.99	1.532	15.01	15.22	1.098
1249	0	0.804	72.37	69.75	15.89	16.16	258.19	1.781	22.25	28.84	1.027
1236	5	0.920	N/D[c]	55.94	20.37	19.42	230.01	1.156	17.03	8.17	1.113
1244	5	0.726	69.98	64.74	18.03	17.14	225.61	1.090	14.99	13.53	0.918
1242	10	0.668	61.05	37.80	15.05	14.69	140.67	0.723	15.93	2.74	0.690
1246	10	0.734	64.16	43.35	16.59	14.80	143.90	0.754	15.10	2.83	1.008
1250	10	0.840	67.04	49.91	18.68	18.63	142.92	0.922	20.41	4.74	1.172
1238	30	0.874	69.58	43.62	15.25	15.61	122.11	0.745	8.17	1.94	0.651
1239	30	Dead	--	--	--	--	--	--	--	--	--
1243	30	0.868	64.02	33.86	11.10	12.50	100.81	0.678	8.15	2.15	0.739
1237	90/45[d]	0.792	78.65	42.62	15.86	18.28	184.52	0.789	14.64	1.49	1.173
1240	90/45	0.804	66.00	38.77	19.21	19.39	136.51	0.802	8.86	2.46	0.783
1241	90/45	0.699	65.19	36.11	14.46	16.03	136.97	0.605	9.17	1.32	0.797
1247	90/45	Dead	--	--	--	--	--	--	--	--	--
1248	90/45	Dead	--	--	--	--	--	--	--	--	--

[a] thyroids, including parathyroids
[b] switched to 5 µg/kg group (Group 5) on Day 8
[c] N/D = no data; brain inadvertently not weighed at necropsy
[d] dose decreased from 90 to 45 µg/kg on Day 8

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-17

Individual Animal Organ-to-Body Weight Ratios[a] - Males

Animal Number	Dose	FBW[b]	Adrenals	Brain	Heart	Kidney (Left)	Kidney (Right)	Liver	Spleen	Testes	Thymus	Thyroid[c]
1252	0	8.60	0.009	0.85	0.85	0.23	0.23	2.91	0.25	0.10	0.16	0.014
1256	0	8.70	0.009	0.78	0.87	0.24	0.24	3.04	0.22	0.10	0.21	0.013
1258	0	Moved[d]	--	--	--	--	--	--	--	--	--	--
1263	0	8.52	0.011	0.85	0.84	0.24	0.24	3.26	0.26	0.07	0.24	0.013
1266	0	Moved	--	--	--	--	--	--	--	--	--	--
1258	5	8.18	0.012	0.91	0.88	0.24	0.24	3.33	0.35	0.04	0.14	0.017
1266	5	8.98	0.010	0.84	0.95	0.26	0.26	2.76	0.27	0.10	0.17	0.020
1257	10	4.86	0.016	1.39	0.86	0.35	0.33	3.89	0.32	0.08	0.07	0.019
1260	10	5.78	0.016	1.25	0.83	0.34	0.32	3.23	0.22	0.03	0.05	0.015
1262	10	4.46	0.023	1.71	1.04	0.58	0.54	4.55	0.42	0.03	0.05	0.017
1259	30	Dead	--	--	--	--	--	--	--	--	--	--
1261	30	Dead	--	--	--	--	--	--	--	--	--	--
1265	30	4.96	0.019	1.39	0.92	0.33	0.31	3.01	0.29	0.03	0.06	0.024
1251	90/45[e]	Dead	--	--	--	--	--	--	--	--	--	--
1253	90/45	Dead	--	--	--	--	--	--	--	--	--	--
1254	90/45	4.74	0.021	1.54	0.93	0.48	0.45	3.19	0.21	0.06	0.04	0.016
1255	90/45	Dead	--	--	--	--	--	--	--	--	--	--
1264	90/45	4.14	0.021	1.56	1.00	0.37	0.36	3.95	0.24	0.04	0.05	0.023

[a] Organ-to-Body Weight Ratio = [Absolute Organ Weight (g) ÷ Final Body Weight (kg)] x 100
[b] FBW = Final Body Weight (kg)
[c] thyroids, including parathyroids
[d] switched to 5 μg/kg group (Group 5) on Day 8
[e] dose decreased from 90 to 45 μg/kg on Day 9

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

Appendix C Table C-17 (cont.)

Individual Animal Organ-to-Body Weight Ratios* - Females

Animal Number	Dose	FBW[b]	Adrenals	Brain	Heart	Kidney (Left)	Kidney (Right)	Liver	Ovaries	Spleen	Thymus	Thyroid[c]
1235	0	7.40	0.012	0.99	0.87	0.24	0.27	3.30	0.019	0.26	0.15	0.012
1236	0	Moved[d]	--	--	--	--	--	--	--	--	--	--
1244	0	Moved	--	--	--	--	--	--	--	--	--	--
1245	0	7.64	0.010	0.90	0.86	0.24	0.24	3.06	0.020	0.20	0.20	0.014
1249	0	8.84	0.009	0.82	0.79	0.18	0.18	2.92	0.020	0.25	0.33	0.012
1236	5	6.68	0.014	N/D[e]	0.84	0.30	0.29	3.44	0.017	0.25	0.12	0.017
1244	5	7.26	0.010	0.96	0.89	0.25	0.24	3.11	0.015	0.21	0.19	0.013
1242	10	4.32	0.015	1.41	0.88	0.35	0.34	3.26	0.017	0.37	0.06	0.016
1246	10	5.02	0.015	1.28	0.86	0.33	0.29	2.87	0.015	0.30	0.06	0.020
1250	10	5.38	0.016	1.25	0.93	0.35	0.35	2.66	0.017	0.38	0.09	0.022
1238	30	4.04	0.022	1.72	1.08	0.38	0.39	3.02	0.018	0.20	0.05	0.016
1239	30	Dead	--	--	--	--	--	--	--	--	--	--
1243	30	3.64	0.024	1.76	0.93	0.30	0.34	2.77	0.019	0.22	0.06	0.020
1237	90/45[f]	4.58	0.017	1.72	0.93	0.35	0.40	4.03	0.017	0.32	0.03	0.026
1240	90/45	3.98	0.020	1.66	0.97	0.48	0.49	3.43	0.020	0.22	0.06	0.020
1241	90/45	4.04	0.017	1.61	0.89	0.36	0.40	3.39	0.015	0.23	0.03	0.020
1247	90/45	Dead	--	--	--	--	--	--	--	--	--	--
1248	90/45	Dead	--	--	--	--	--	--	--	--	--	--

[a] Organ-to-Body Weight Ratio = [Absolute Organ Weight (g) ÷ Final Body Weight (kg)] x 100
[b] FBW = Final Body Weight (kg)
[c] thyroids, including parathyroids
[d] switched to 5 μg/kg group (Group 5) on Day 8
[e] N/D = no data; brain inadvertently not weighed at necropsy
[f] dose decreased from 90 to 45 μg/kg on Day 8

Appendix D. Ophthalmology Report

ANIMAL EYE ASSOCIATES

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Ophthalmic Exam Report
Project No. 1209/SN2
March 21, 2001

Pre-study ophthalmic examinations were performed on 8/24/00 according to SOP standards. Two animals were found to have ophthalmic variations of normal and retained in the study (permanent number female 1244 and permanent number male 1265). One animal (permanent number male 1262) had a corneal opacity OS, which did not preclude examination of the fundus. The animal was retained in the study.

Post-treatment ophthalmic examinations were performed on 9/28/00 according to SOP standards. One animal (permanent number female 1241) had a corneal ulcer OD which precluded examination of intraocular structures. In my opinion, the ulceration was not test article related. There was no change in the ophthalmic variations of normal in permanent number female1244 or permanent number male 1265. The corneal opacity in permanent number male 1262 had resolved. All remaining test animals were within normal limits acceptable for this breed, age, and housing conditions.

A L Hunkeler
Amy L. Hunkeler D.V.M.

Appendix E. Electrocardiography Report

Appendix G. Histopathology Report

DRAFT PATHOLOGY REPORT FOR
FOUR WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS
IITRI PROJECT NUMBER 1209 STUDY NUMBER 2

PREPARED
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MARCH 20, 2001

TABLE OF CONTENTS

SECTION I

PATHOLOGY NARRATIVE

DRAFT PATHOLOGY REPORT

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF 1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

INTRODUCTION

This pathology report, submitted by Pathology Associates to IIT Research Institute (IITRI), represents the histopathology findings for the study designated as "Four-Week Oral (Gavage) Toxicity Study of 1α-Hydroxyvitamin D_5 In Beagle Dogs," IITRI Project Number 1209, Study Number 2.

The study was conducted to evaluate the toxicity of 1α-Hydroxyvitamin D_5 when administered orally to beagle dogs for four weeks.

EXPERIMENTAL DESIGN AND METHODS

Four groups [(groups 2-5), group 2 and group 3 composed of 3 male and 3 female, group 4 composed of 5 male and 5 female, and group 5 composed of 2 male and 2 female Beagle dogs] were given the test article once daily by oral gavage in 1 ml/kg body weight of test article vehicle (corn oil) for a minimum of 28 days. The dose levels administered were 10, 30, 45 (decreased from 90 μg/kg on study day 9 and 8 for males and females, respectively), and 5 μg/kg body weight for animals in groups 2, 3, 4, and 5, respectively. Also, one group (group 1), composed of 3 male and 3 female Beagle dogs was given the test article vehicle alone daily by oral gavage for a minimum of 28 days. The experimental design is summarized in Table I (Summary of Experimental Design).

Several animals (2 high dose males, 2 high dose females, and 1 high-mid dose male) died prior to the end of the study. All terminal sacrifice animals were sacrificed and necropsied on study day 29-37. Terminal sacrifice and moribund sacrificed animals were euthanized by barbituate overdose. All necropsies were performed according to IITRI Standard Operating Procedures and were conducted by Pathology Associates personnel. Tissues required by the protocol (see Table II, Protocol-Required Tissues) were examined and placed in 10% neutral buffered formalin.

Tissues required for histopathologic evaluation in groups 1, 2, 5, and group 3 moribund sacrificed animals (animal numbers 1261 and 1239) were trimmed and processed, and slides were prepared in accordance with Pathology Associates Standard Operating Procedures. These tissues were evaluated by light microscopy and the results were tabulated. Some tissues are inherently difficult to obtain in sections because of their small size (e.g. parathyroid gland). Tissues were recorded as "unavailable/unsuitable for complete evaluation" when they were missing in both the original section and in recut and/or retrim attempts to obtain them.

Treatment-related lesions are summarized in Table III, Summary of Treatment-Related Lesions. Microscopic findings for all groups are summarized in the Project Summary tables (Section II). The

mean group severity scores (SEV) are found in the Severity Summary tables (Section III). Where applicable, all tissue changes received a severity grade based upon the following scale: 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked. The mean group severity was determined by dividing the sum of the severity scores by the number of tissues in the group. Microscopic findings in the protocol-required tissues for individual animals are presented in the Tabulated Animal Data tables (Section IV). The correlation of the necropsy findings and histopathology findings are reported in the Correlation of Gross and Microscopic (Micro) Findings (Section V). The codes used as entries in these tables are explained in the Report Codes Table.

The portion of this study performed by Pathology Associates was conducted in compliance with the US Food and Drug Administration's Good Laboratory Practice (GLP) Regulations for Nonclinical Laboratory Studies, 21 CFR Part 58.

RESULTS AND DISCUSSION

The Results and Discussion section is divided into three parts: Necropsy Findings, Diagnostic Terms, and Histopathology Findings. The Necropsy Findings portion describes lesions seen at necropsy or trimming. The Diagnostic Terms portion lists and clarifies diagnostic terminology that may be unclear. Terms listed in the Diagnostic Terms portion of this section include, but are not limited to, those that are considered to be test article-related. The Histopathology Findings portion of this section reports the results and provides discussion of the histopathologic evaluation of the tissues.

Necropsy Findings

Early deaths were observed in 3 of the high dose males [one found dead on study day 23 (animal number 1255), one found dead on study day 27 (animal number 1251), and one moribund sacrificed on study day 23 (animal number 1253)], in 2 of the high dose females [one found dead on study day 6 (animal number 1248) and one found dead on study day 7 (animal number 1247)], in 2 high-mid dose males [one found dead on study day 24 (animal number 1259) and one moribund sacrificed on study day 24 (animal number 1261)], and in one high-mid dose female [moribund sacrificed on study day 28 (animal number 1239)].

Gross necropsy observations are summarized in Table IV (Summary of Gross Necropsy Observations). Pigmentation changes in the lung, kidney, stomach, spleen, and intestine were more commonly present in groups 2, 3, and 4 compared to groups 1 and 5. Small thymus was observed in all dogs in groups 2, 3, and 4 and correlated with a microscopic diagnosis of atrophy.

All other gross lesions were interpreted as incidental findings. Gross observations are listed in the Correlation of Gross and Microscopic (Micro) Findings report in Section V. Microscopic findings for animals evaluated microscopically were correlated with gross lesions when possible.

Diagnostic Terms

The morphologic characteristics of observations and lesions which require comment are presented in subsequent paragraphs to aid in the interpretation of the data.

Kidney

Renal lesions occurred as uniform rays of basophilic staining distal convoluted tubules within relatively normal proximal convoluted tubules and glomeruli. The rays of tissue were characterized by the presence of dilated basophilic staining distal convoluted tubules with thin attenuated epithelium in the outer cortex and the presence of foci of deeply basophilic or amphoteric granular material (mineralization) in the lumen of basophilic staining distal convoluted tubules or collecting ducts in the inner cortex. The presence of tubule dilatation, mineralization, and basophilic staining of tubules were diagnosed separately to distinguish these generalized changes from similar findings that occasionally occur (focal nephropathy) as an incidental finding.

Stomach

Mid-zonal mineralization was characterized by the presence of foci of deeply basophilic or amphoteric granular material in the mid-zonal region of the pyloric stomach mucosa in minimal lesions. In more advanced mid-zonal mineralization, most epithelial cells in the mid-zonal region contain deeply basophilic granular material.

Bone, Femur

Hypoplasia of epiphyseal cartilage was characterized by decreased thickness of the epiphyseal plate, reduced size and number of trabeculae on the diaphyseal side of the epiphyseal disk, increased thickness of trabeculae, and eosinophilic staining of the intercellular substance of young proliferating cartilage.

Bone Marrow

Depletion of bone marrow was characterized by decreased cellularity due to replacement of hematopoietic cells with fat cells.

Thymus

Atrophy was characterized by reduced size of thymic lobules, mainly due to a lack of cortical lymphoid tissue.

Heart

Mineralization at the aortic base was characterized by loss of normal architecture and the presence of deeply basophilic or amphoteric granular material in the arterial wall.

Salivary Gland

Necrosis of parotid salivary gland was characterized by focal loss of normal architecture and the presence of cell debris. Mineralization was characterized by the presence of foci of deeply basophilic material. Some of the foci consisted of concentric rings of basophilic material of variable density.

Skeletal Muscle

Atrophy was characterized by decreased average diameter of muscle fibers. Degeneration with associated subacute inflammation was characterized by replacement of deeply

eosinophilic homogeneous muscle fibers with lightly eosinophilic finely vacuolated material mixed with neutrophils and macrophages.

Skin

Abscess was characterized by the focal presence of neutrophils and cell debris in the subcutis. Ulceration was characterized by the loss of the epithelium and with replacement by cell debris and neutrophils.

Spleen

Arterial mineralization was characterized by the presence of focal regions of deeply basophilic granular material in the muscular wall of large arteries.

Thyroid Gland

Hypertrophy/hyperplasia of parafollicular cells was characterized by an increased ratio of parafollicular cells relative to follicular cells and the presence of foci of parafollicular cells that were increased in size due to an increased amount of finely vacuolated lightly basophilic cytoplasm.

Parathyroid Gland

Hypertrophy was characterized by diffusely increased cell size due to an increased amount of finely vacuolated lightly basophilic cytoplasm.

Uterus

Atrophy was characterized by notably reduced uterine wall thickness and overall cross-sectional diameter of uterus relative to the control animals.

Adrenal Gland

Mineralization was characterized by the presence of a single focus of inner cortex wherein the normal architecture was altered by the presence of deeply basophilic or amphoteric granular material. Vacuolation was characterized by the focal presence of individual cortical cells that are markedly enlarged due to the presence of multiple large vacuoles in their cytoplasm.

The remainder of the diagnoses used in this study were considered to be self-explanatory and were not discussed in this section.

Histopathology Findings

The incidence and severity of treatment-related histopathology findings are summarized in Table III, Summary of Treatment-Related Lesions. These findings are further discussed by organ in this section of the narrative report.

Kidney

Tubule dilatation was observed in the high-mid dose male (SEV = 3.00), the low-mid dose males (3/3, SEV = 3.00), the low dose males (1/2, SEV = 1.00), the high-mid dose female (SEV = 3.00), the low-mid dose females (3/3, SEV = 3.33), and the low dose females (1/2, SEV = 0.50). Cortical mineralization was observed in the high-mid dose male (SEV = 3.00),

the low-mid dose males (3/3, SEV = 2.00), the low dose males (1/2, SEV = 0.50), the high-mid dose female (SEV = 3.00), the low-mid dose females (3/3, SEV = 2.00), and the low dose females (1/2, SEV = 0.50). Diffuse basophilic tubules were observed in the high-mid dose male (SEV = 3.00), the low-mid dose male (3/3, SEV = 3.00), the low dose males (1/2, SEV = 1.00), the high-mid dose female (SEV = 3.00), the low-mid dose females (3/3, SEV = 3.00), and the low dose females (1/2, SEV = 0.50). Tubule dilatation, cortical mineralization, and diffuse basophilic tubules were interpreted as treatment-related findings.

Stomach

Mineralization of mid-mucosal region of pyloric stomach was observed in the high-mid dose male (SEV = 3.00), the low-mid dose males (1/3, SEV = 1.00), the low dose males (1/2, SEV = 0.50), the high-mid dose female (SEV = 4.00), the low-mid dose females (2/3, SEV = 1.33), and the low-dose females (1/2, SEV = 1.00). Mineralization of mid-mucosal region of stomach was interpreted as a treatment-related finding.

Bone, Femur

Hypoplasia of epiphyseal cartilage was observed in the high-mid dose male (SEV = 2.00), the low-mid dose males (3/3, SEV = 2.00), the high-mid dose female (SEV = 2.00), and the low-mid dose females (3/3, SEV = 2.00). Hypoplasia of epiphyseal cartilage was interpreted as a treatment-related finding.

Bone Marrow, Femoral

Depletion of bone marrow in femur was observed in the low-mid dose males (3/3, SEV = 2.67), the high-mid dose female (SEV = 3.00), and the low-mid dose females (3/3, SEV = 2.33). Bone marrow depletion was interpreted as a treatment-related finding.

Bone Marrow, Sternum

Depletion of bone marrow in sternum was observed in the low-mid dose males (2/3, SEV = 1.00), the high-mid dose female (SEV = 3.00), and the low-mid dose females (2/3, SEV = 0.67). Bone marrow depletion in sternum was interpreted as a treatment-related finding. The lack of bone marrow depletion in the high-mid dose male sacrificed on study day 24 (animal number 1261) may be related to the presence of extensive skin ulceration in that animal.

Thymus

Atrophy was observed in the high-mid dose male (SEV = 4.00), the low-mid dose males (3/3, SEV = 3.33), the high-mid dose female (SEV = 4.00), the low-mid dose females (3/3, SEV = 2.67), and the low dose females (2/2, SEV = 1.50). Thymic atrophy was interpreted as a treatment-related finding.

Heart

Mineralization of aortic muscle wall at the aortic base of heart was observed in the high-mid dose male (SEV = 3.00) and the low-mid dose males (1/3, SEV = 0.67). Mineralization at the aortic base was interpreted as a treatment-related finding.

Skeletal Muscle

Atrophy was observed in the high-mid dose male (SEV = 3.00), the low-mid dose males (3/3, SEV = 2.00), the high-mid dose female (SEV = 3.00), and the low-mid dose females (3/3, SEV = 2.00). Degeneration was observed in the high-mid dose female (SEV = 3.00). Subacute inflammation was observed in the high-mid dose female (SEV = 2.00). Atrophy, degeneration, and subacute inflammation of muscle were interpreted as treatment-related findings. However, the subacute inflammation was interpreted as secondary to the muscle degeneration.

Spleen

Mineralization of splenic artery wall was observed in the high-mid dose male (SEV = 2.00) and the low-mid dose females (2/3, SEV = 1.00). Mineralization of splenic artery was interpreted as a treatment-related finding.

Thyroid Gland

Hypertrophy/hyperplasia of parafollicular cells was observed in the high-mid dose male (SEV = 3.00), the low-mid dose males (3/3, SEV = 1.67), the high-mid dose female (SEV = 3.00), the low-mid dose females (2/3, SEV = 0.67), and the low dose females (1/2, SEV = 0.50). Hypertrophy/hyperplasia of parafollicular cells was interpreted as a treatment-related finding, but it was considered to be a secondary metabolic effect of minimal toxicological significance.

Parathyroid Gland

Hypertrophy was observed in the high-mid dose male (SEV = 2.00) and the low-mid dose females (3/3, SEV = 1.00). Hypertrophy of parathyroid glands was interpreted as an equivocal finding that may represent slightly increased storage in response to persistent hypercalcemia, or a direct response to vitamin D metabolites.

Uterus

Atrophy was observed in the high-mid dose female (SEV = 3.00) and the low-mid dose females (3/3, SEV = 2.33). Atrophy was interpreted as a treatment-related finding, but was probably secondary to the generalized weight loss and debility of the animals.

Adrenal Gland

Focal mineralization of adrenal cortex was observed in the low-mid dose females (1/3, SEV = 0.67). Vacuolation of adrenal cortex was observed in the high-mid dose female (SEV = 2.00). Focal mineralization and vacuolation were interpreted as equivocal findings. These lesions can occasionally occur as incidental findings, but they are not common.

Skin

Abscess was observed in the low-mid dose males (1/3, SEV = 1.33). Ulceration was observed in the high-mid dose male (SEV = 4.00) and the low-mid dose males (1/3, SEV = 1.33). Abscessation and ulceration of skin were interpreted as treatment-related findings that are probably secondary to uremia from the renal lesions.

Salivary Gland

Focal necrosis and associated mineralization were observed in parotid salivary gland from one low-mid dose male (animal number 1262). The parotid gland was incidentally present with the submandibular salivary gland routinely sampled.

All other microscopic findings were interpreted as incidental findings that are commonly present in dog toxicology studies.

CONCLUSIONS

Under the conditions of this study, daily administration of 1α-Hydroxyvitamin D_5 by oral gavage to Beagle dogs for a minimum of 28 days at a dose of 90/45 or 30 μg/kg body weight resulted in early deaths (found dead or moribund sacrificed). Similar administration of 1 α-Hydroxyvitamin D_5 at a dose of 10 or 5 μg/kg resulted in significant renal toxicity (tubule dilatation, cortical mineralization, and basophilic tubules), mid-mucosal pyloric mineralization in stomach, thymic atrophy (females only at 5 μg/kg), and hypertrophy/hyperplasia of thyroid parafollicular cells (females only at 5 μg/kg). Administration of 1α-Hydroxyvitamin D_5 at a dose of 10 μg/kg also resulted in mineralization in arteries of spleen (females only) and heart (males only), bone marrow depletion, cartilage hypoplasia in femur, and skeletal muscle atrophy.

A histopathology no-effect level was not attained in this study. However, only kidneys, stomach, thymus (females only), and thyroid parafollicular cells (females only) were affected in animals given the 5 μg/kg dose of 1α-Hydroxyvitamin D_5.

Robert L. Morrissey, DVM, Ph.D.
Diplomate, ACVP

Date

TABLE I
SUMMARY OF EXPERIMENTAL DESIGN

Group Number	Group	Dose Level (µg/kg body weight)	Number of Males	Number of Females
1	Control	0	3	3
5	Low	5	2	2
2	Low-Mid	10	3	3
3	High-Mid	30	3	3
4	High	90/45	5	5

TABLE II
PROTOCOL-REQUIRED TISSUES

Adrenal glands
Aorta (thoracic)
Brain (entire)
Cecum
Colon
Diaphragm
Duodenum (with bile and pancreatic ducts)
Epididymides
Esophagus
Eyes with optic nerves
Femur, including diaphysis with marrow cavity and epiphysis (femoral condyle with epiphyseal cartilage plate, articular cartilage, and articular surface)
Gall bladder
Heart
Ileum
Jejunum
Kidneys
Liver (right medial and left lateral lobes)
Lungs (left apical [infused] and left diaphragmatic [non-infused] lobes) and Bronchi
Lymph nodes (bronchial, mandibular, mesenteric)
Mammary gland (left inguinal, with skin)
Ovaries and fallopian tubes
Pancreas
Pituitary gland
Prostate
Rectum
Salivary gland (mandibular)
Sciatic nerve
Skeletal muscle
Skin (dorsal thorax, elbow)
Spinal cord (cervical, thoracic)
Spleen
Sternum with bone marrow
Stomach (fundic, and pyloric regions)
Testes
Thymus
Thyroid gland with parathyroids
Tongue
Tonsil (palatine)
Trachea
Ureter
Urinary bladder
Uterus (corpus and cervix)
Vagina
Gross lesions
Tissue masses and regional lymph nodes

TABLE III

SUMMARY OF TREATMENT-RELATED LESIONS

ORGAN - lesion		Dose (ug/kg body weight) 0	5	10	30
KIDNEY - Dilatation, tubules	M	0/3	1/2 (1.00)	3/3 (3.00)*	1/1 (3.00)
	F	0/3	1/2 (0.50)	3/3 (3.33)	1/1 (3.00)
- Mineralization, cortex	M	0/3	1/2 (0.50)	3/3 (2.00)	1/1 (3.00)
	F	0/3	1/2 (0.50)	3/3 (2.00)	1/1 (3.00)
- Basophilic tubules, diffuse	M	0/3	1/2 (1.00)	3/3 (3.00)	1/1 (3.00)
	F	0/3	1/2 (0.50)	3/3 (3.00)	1/1 (3.00)
STOMACH - Mineralization, mid-mucosal, pyloric	M	0/3	1/2 (0.50)	1/3 (1.00)	1/1 (3.00)
	F	0/3	1/2 (1.00)	2/3 (1.33)	1/1 (4.00)
BONE, FEMUR - Hypoplasia, epiphyseal cartilage	M	0/3	0/2	3/3 (2.00)	1/1 (2.00)
	F	0/3	0/2	3/3 (2.00)	1/1 (2.00)
BONE MARROW, FEMORAL - Depletion	M	0/3	0/2	3/3 (2.67)	0/1
	F	0/3	0/2	3/3 (2.33)	1/1 (3.00)
BONE MARROW, STERNUM - Depletion	M	0/3	0/2	2/3 (1.00)	0/1
	F	0/3	0/2	2/3 (0.67)	1/1 (3.00)
THYMUS - Atrophy	M	0/3	0/2	3/3 (3.33)	1/1 (4.00)
	F	0/3	2/2 (1.50)	3/3 (2.67)	1/1 (4.00)
HEART - Mineralization, aortic base	M	0/3	0/2	1/3 (0.67)	1/1 (3.00)
	F	0/3	0/2	0/3	0/1
SPLEEN - Mineralization, artery	M	0/3	0/2	0/3	1/1 (2.00)
	F	0/3	0/2	2/3 (1.00)	0/1
THYROID GLAND - Hypertrophy/hyperplasia, parafollicular cell	M	0/3	0/2	3/3 (1.67)	1/1 (3.00)
	F	0/3	1/2 (0.50)	2/3 (0.67)	1/1 (3.00)
PARATHYROID GLAND - Hypertrophy	M	0/3	0/2	0/3	1/1 (2.00)
	F	0/3	0/2	3/3 (1.00)	0/1

* Incidence (mean group severity score)

TABLE III
SUMMARY OF TREATMENT-RELATED LESIONS

		Dose (ug/kg body weight)			
ORGAN - lesion		0	5	10	30
SKELETAL MUSCLE					
- Atrophy	M	0/3	0/2	3/3 (2.00)	1/1 (3.00)
	F	0/3	0/2	3/3 (2.00)	1/1 (3.00)
- Degeneration	M	0/3	0/2	0/3	0/1
	F	0/3	0/2	0/3	1/1 (3.00)
- Inflammation, subacute	M	0/3	0/2	0/3	0/1
	F	0/3	0/2	0/3	1/1 (2.00)
SKIN					
- Abscess	M	0/3	0/0	1/3 (1.33)	0/1
	F	0/3	0/1	0/3	0/1
- Ulceration	M	0/3	0/0	1/3 (1.33)	1/1 (4.00)
	F	0/3	0/1	0/3	0/1
ADRENAL GLAND					
- Mineralization, cortex, focal	M	0/3	0/2	0/3	0/1
	F	0/3	0/2	1/3 (0.67)	0/1
- Vacuolation, cortex	M	0/3	0/2	0/3	0/1
	F	0/3	0/2	0/3	1/1 (2.00)
UTERUS					
- Atrophy	F	0/3	0/0	3/3 (2.33)	1/1 (3.00)

* Incidence (mean group severity score)

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

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TABLE IV

Summary of Gross Necropsy Observations

Tissue/Lesion	Group 1 M	Group 1 F	Group 5 M	Group 5 F	Group 2 M	Group 2 F	Group 3 M	Group 3 F	Group 4 M	Group 4 F
Lymph node, mandibular										
pigmentation	--[a]	--	--	--	1	--	--	1	--	--
enlarged	--	--	--	--	1	--	1	--	1	--
Lymph node, bronchial pigmentation	1	1	--	--	--	--	--	1	--	2
Lymph node, mediastinal pigmentation	2	2	--	--	--	1	--	--	2	2
Lymph node, mesenteric pigmentation	1	--	--	--	--	--	--	1	--	1
Lymph node, deep cervical										
pigmentation	--	--	--	--	1	--	--	--	--	--
enlarged	--	--	--	--	1	--	--	--	--	--
Lung										
pigmentation	--	--	--	--	--	--	1	2	5	5
focus	--	--	--	--	--	--	3	--	--	3
mass	--	--	--	--	--	--	--	--	2	--
Kidney										
pigmentation	--	--	--	--	3	2	1	1	1	1
dilatation	--	--	--	--	1	--	--	--	--	--
Stomach										
pigmentation	--	--	--	--	--	--	1	1	6	3
focus	--	--	--	--	--	--	3	--	--	2

--[a] = no signs observed

Group 1 = 0 µg/kg body weight
Group 5 = 5 µg/kg body weight
Group 2 = 10 µg/kg body weight
Group 3 = 30 µg/kg body weight
Group 4 = 90/45 µg/kg body weight

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

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TABLE IV (cont.)

Summary of Gross Necropsy Observations

Tissue/Lesion	Group 1 M	Group 1 F	Group 5 M	Group 5 F	Group 2 M	Group 2 F	Group 3 M	Group 3 F	Group 4 M	Group 4 F
Spleen										
Pigmentation	--[a]	--	--	--	--	--	--	--	2	2
Focus	--	--	--	--	--	--	--	--	1	--
Thymus										
Pigmentation	--	--	--	1	--	--	--	--	1	1
Small	--	--	--	--	3	3	3	3	5	3
Small intestine, duodenum										
Pigmentation	--	--	--	--	1	3	3	2	4	5
Small intestine, jejunum										
Pigmentation	--	--	--	--	1	1	3	3	4	1
Small intestine, ileum										
Focus	--	--	--	--	--	--	1	--	--	--
Pigmentation	--	--	1	--	1	1	1	1	--	--
Large intestine, cecum										
Pigmentation	--	--	1	--	1	--	--	1	2	1
Large intestine, colon										
Pigmentation	--	--	--	--	1	--	2	2	2	1
Large intestine, rectum										
Pigmentation	--	--	--	--	1	1	2	2	3	5
Tongue										
Pigmentation	--	--	--	--	--	--	1	--	--	--
Tonsil										
Pigmentation	1	--	2	--	3	--	2	1	1	1
Thyroid gland										
Pigmentation	--	--	--	--	--	--	--	--	2	3

--[a] = no signs observed

Group 1 = 0 μg/kg body weight
Group 5 = 5 μg/kg body weight
Group 2 = 10 μg/kg body weight
Group 3 = 30 μg/kg body weight
Group 4 = 90/45 μg/kg body weight

FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS

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TABLE IV (cont.)

Summary of Gross Necropsy Observations

Tissue/Lesion	Group 1 M	Group 1 F	Group 5 M	Group 5 F	Group 2 M	Group 2 F	Group 3 M	Group 3 F	Group 4 M	Group 4 F
Prostate										
Small	3	--[a]	2	--	3	--	1	--	3	--
Bone										
Lesion	--	--	1	--	--	--	--	--	--	--
Mesentery										
nodule	--	--	--	--	--	1	--	--	--	--
Eye										
pigmentation	--	--	--	--	--	--	--	--	--	1
Skin										
pigmentation	--	--	--	--	--	--	2	--	--	--
Thick	--	--	--	--	1	--	--	--	1	--
Epididymis										
Small	--	--	--	--	3	--	2	--	3	--
Testes										
Small	--	--	1	--	3	--	2	--	3	--
Ovary										
small	--	--	--	--	--	--	--	2	--	--
Uterus										
small	--	--	--	--	--	3	--	3	--	3

--[a] = no signs observed

Group 1 = 0 μg/kg body weight
Group 5 = 5 μg/kg body weight
Group 2 = 10 μg/kg body weight
Group 3 = 30 μg/kg body weight
Group 4 = 90/45 μg/kg body weight

PATHOLOGY ASSOCIATES INTERNATIONAL
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1α-HYDROXYVITAMIN D_5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209, STUDY NUMBER 2

Report Codes Table

A. Codes applying to organs

N	Tissues within normal histological limits
A	Autolysis precluding adequate evaluation
U	Tissues unavailable/unsuitable for complete evaluation

B. Codes applying to microscopic diagnoses

1	minimal
2	mild
3	moderate
4	marked
( )	focal
[ ]	diffuse
< >	multifocal
P	Present
I	Bilateral
L	Unilateral
-	No data entered

SECTION II

PROJECT SUMMARY

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

PROJECT SUMMARY

STUDY ID : 1209 SN2 STUDY NUMBER: 1209SN2
FATE: ALL
DAYS ON TEST: ALL SEX: MALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1 (1)		5 (2)		2 (3)		3 (4)	
NUMBER OF ANIMALS:		3		2		3		3	
		#	%	#	%	#	%	#	%
BRAIN, FORE	# EX	3		0		3		1	
Hemorrhage, acute, perivascular		0	0.0	0	0.0	1	33.3	0	0.0
SPINAL CORD, CERVICAL	# EX	3		0		3		1	
BRAIN, MID	# EX	3		0		3		1	
Mineralization, meninges		0	0.0	0	0.0	0	0.0	1	100.0
SPINAL CORD, THORACIC	# EX	3		0		3		1	
BRAIN, HIND	# EX	3		0		3		1	
HEART	# EX	3		2		3		1	
Mineralization, aortic base		0	0.0	0	0.0	1	33.3	1	100.0
TRACHEA	# EX	3		0		3		1	
Inflammation, subacute		1	33.3	0	0.0	0	0.0	0	0.0
Mineralization, focal		0	0.0	0	0.0	0	0.0	1	100.0
ESOPHAGUS	# EX	3		0		3		1	
AORTA	# EX	3		0		3		1	
LYMPH NODE, BRONCHIAL	# EX	3		0		3		1	
Sinus erythrocytosis		2	66.7	0	0.0	0	0.0	0	0.0
Depletion, lymphoid		0	0.0	0	0.0	0	0.0	1	100.0
LUNG	# EX	3		0		3		1	
Inflammation, subacute, focal		2	66.7	0	0.0	1	33.3	0	0.0
Inflammation, chronic, perivascular		2	66.7	0	0.0	1	33.3	1	100.0
Hemorrhage, acute, focal		1	33.3	0	0.0	0	0.0	0	0.0

Incidence Calculated by No. of Tissues Scored
(1) - 0 ug/kg body weight
(2) - 5 ug/kg body weight
(3) - 10 ug/kg body weight
(4) - 30 ug/kg body weight

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PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

PROJECT SUMMARY

STUDY ID : 1209 SN2 — STUDY NUMBER: 1209SN2
FATE: ALL
DAYS ON TEST: ALL — SEX: MALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1 (1)		5 (2)		2 (3)		3 (4)	
NUMBER OF ANIMALS:		3		2		3		3	
		#	%	#	%	#	%	#	%
LUNG	# EX	3		0		3		1	
Inflammation, granulomatous, focal		0	0.0	0	0.0	1	33.3	0	0.0
KIDNEY	# EX	3		2		3		1	
Mineralization, medulla		3	100.0	2	100.0	3	100.0	1	100.0
Basophilic tubules		1	33.3	0	0.0	0	0.0	0	0.0
Dilatation, tubules		0	0.0	1	50.0	3	100.0	1	100.0
Mineralization, cortex		0	0.0	1	50.0	3	100.0	1	100.0
Basophilic tubules, diffuse		0	0.0	1	50.0	3	100.0	1	100.0
Congestion		0	0.0	0	0.0	3	100.0	0	0.0
Dilatation, pelvis		0	0.0	0	0.0	1	33.3	0	0.0
Inflammation, chronic		0	0.0	2	100.0	0	0.0	1	100.0
SMALL INTESTINE,DUODENUM	# EX	3		0		3		1	
Dilatation, mucosal gland		1	33.3	0	0.0	1	33.3	1	100.0
Congestion		0	0.0	0	0.0	0	0.0	1	100.0
SPLEEN	# EX	3		2		3		1	
Mineralization, artery		0	0.0	0	0.0	0	0.0	1	100.0
PANCREAS	# EX	3		0		3		1	
LYMPH NODE,MESENTERIC	# EX	3		0		3		1	
Sinus erythrocytosis		3	100.0	0	0.0	1	33.3	0	0.0
Depletion, lymphoid		0	0.0	0	0.0	0	0.0	1	100.0
LIVER	# EX	3		0		3		1	
Inflammation, chronic, periportal		2	66.7	0	0.0	2	66.7	1	100.0
Inflammation, granulomatous, focal		0	0.0	0	0.0	1	33.3	0	0.0
Inflammation, chronic, focal		0	0.0	0	0.0	1	33.3	0	0.0

Incidence Calculated by No. of Tissues Scored
(1) - 0 ug/kg body weight
(2) - 5 ug/kg body weight
(3) - 10 ug/kg body weight
(4) - 30 ug/kg body weight

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PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

PROJECT SUMMARY

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2

SEX: MALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1 (1)		5 (2)		2 (3)		3 (4)	
NUMBER OF ANIMALS:		3		2		3		3	
		#	%	#	%	#	%	#	%
GALLBLADDER	# EX	3		0		3		1	
Accumulation, lymphocyte		3	100.0	0	0.0	0	0.0	0	0.0
LARGE INTESTINE,RECTUM	# EX	3		0		3		1	
Dilatation, crypt glands		0	0.0	0	0.0	1	33.3	0	0.0
Congestion		0	0.0	0	0.0	1	33.3	1	100.0
ADRENAL GLAND	# EX	3		2		3		1	
PERIPHERAL NERVE,SCIATIC	# EX	3		0		3		1	
SALIVARY GLAND	# EX	3		2		3		1	
Necrosis, focal, parotid		0	0.0	0	0.0	1	33.3	0	0.0
Mineralization, focal, parotid		0	0.0	0	0.0	1	33.3	0	0.0
TONGUE	# EX	3		0		3		1	
Inflammation, chronic, perivascular		1	33.3	0	0.0	1	33.3	0	0.0
Inflammation, subacute, focal		0	0.0	0	0.0	1	33.3	0	0.0
Erosion, focal		0	0.0	0	0.0	1	33.3	0	0.0
LYMPH NODE,MANDIBULAR	# EX	3		0		3		1	
Sinus erythrocytosis		2	66.7	0	0.0	1	33.3	1	100.0
Tattoo pigment		0	0.0	0	0.0	1	33.3	0	0.0
SKIN,ELBOW	# EX	3		2		3		1	
Inflammation, subacute, dermis		0	0.0	1	50.0	0	0.0	0	0.0
SMALL INTESTINE,JEJUNUM	# EX	3		0		3		1	
Dilatation, crypt glands		0	0.0	0	0.0	0	0.0	1	100.0
Congestion		0	0.0	0	0.0	0	0.0	1	100.0

Incidence Calculated by No. of Tissues Scored
(1) - 0 ug/kg body weight
(2) - 5 ug/kg body weight
(3) - 10 ug/kg body weight
(4) - 30 ug/kg body weight

LABCAT HP4.33

19-JAN-2001

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

PROJECT SUMMARY

STUDY ID : 1209 SN2 STUDY NUMBER: 1209SN2
FATE: ALL
DAYS ON TEST: ALL SEX: MALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1 (1)		5 (2)		2 (3)		3 (4)	
NUMBER OF ANIMALS:		3		2		3		3	
		#	%	#	%	#	%	#	%
LARGE INTESTINE, COLON	# EX	3		0		3		1	
Dilatation, crypt glands		0	0.0	0	0.0	1	33.3	1	100.0
Congestion		0	0.0	0	0.0	1	33.3	1	100.0
TONSIL	# EX	3		2		3		1	
Mineralization, focal		3	100.0	2	100.0	3	100.0	1	100.0
Inflammation, subacute		3	100.0	2	100.0	3	100.0	1	100.0
Hemorrhage		3	100.0	2	100.0	3	100.0	1	100.0
SKIN, DORSAL THORAX	# EX	3		2		3		1	
SMALL INTESTINE, ILEUM	# EX	3		1		3		1	
Congestion		0	0.0	0	0.0	0	0.0	1	100.0
THYMUS	# EX	3		2		3		1	
Atrophy		0	0.0	0	0.0	3	100.0	1	100.0
SKELETAL MUSCLE	# EX	3		2		3		1	
Atrophy		0	0.0	0	0.0	3	100.0	1	100.0
SKIN	# EX	3		0		3		1	
Abscess		0	0.0	0	0.0	1	33.3	0	0.0
Bacteria		0	0.0	0	0.0	1	33.3	0	0.0
Ulceration		0	0.0	0	0.0	1	33.3	1	100.0
MAMMARY GLAND	# EX	3		0		0		1	
THYROID GLAND	# EX	3		2		3		1	
Hypertrophy/hyperplasia, parafollicular cell		0	0.0	0	0.0	3	100.0	1	100.0

Incidence Calculated by No. of Tissues Scored
(1) - 0 ug/kg body weight
(2) - 5 ug/kg body weight
(3) - 10 ug/kg body weight
(4) - 30 ug/kg body weight

LABCAT HP4.33 19-JAN-2001

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PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

PROJECT SUMMARY

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2

SEX: MALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1 (1)		5 (2)		2 (3)		3 (4)	
NUMBER OF ANIMALS:		3		2		3		3	
		#	%	#	%	#	%	#	%
PARATHYROID GLAND	# EX	3		2		3		1	
Cyst		1	33.3	1	50.0	1	33.3	1	100.0
Hypertrophy		0	0.0	0	0.0	0	0.0	1	100.0
PITUITARY GLAND	# EX	3		0		3		1	
Cyst		0	0.0	0	0.0	2	66.7	0	0.0
URETER	# EX	3		0		3		1	
STOMACH	# EX	3		2		3		1	
Mineralization, focal		1	33.3	0	0.0	0	0.0	0	0.0
Accumulation, lymphocyte		1	33.3	2	100.0	1	33.3	1	100.0
Mineralization, mid-mucosal, pyloric		0	0.0	1	50.0	1	33.3	1	100.0
LARGE INTESTINE,CECUM	# EX	3		1		3		1	
Dilatation, crypt gland		0	0.0	0	0.0	1	33.3	0	0.0
Congestion		0	0.0	0	0.0	1	33.3	0	0.0
URINARY BLADDER	# EX	3		0		3		1	
TESTES	# EX	3		2		3		1	
Sexual immaturity		2	66.7	1	50.0	3	100.0	1	100.0
EPIDIDYMIS	# EX	3		0		3		1	
Oligospermia		2	66.7	0	0.0	3	100.0	1	100.0
PROSTATE	# EX	3		2		3		1	
Sexual immaturity		3	100.0	2	100.0	3	100.0	1	100.0
EYE	# EX	3		0		3		1	

Incidence Calculated by No. of Tissues Scored
(1) - 0 ug/kg body weight
(2) - 5 ug/kg body weight
(3) - 10 ug/kg body weight
(4) - 30 ug/kg body weight

LABCAT HP4.33

19-JAN-2001

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FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

PROJECT SUMMARY

STUDY ID : 1209 SN2
STUDY NUMBER: 1209SN2
FATE: ALL
DAYS ON TEST: ALL
SEX: MALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1 (1)		5 (2)		2 (3)		3 (4)	
NUMBER OF ANIMALS:		3		2		3		3	
		#	%	#	%	#	%	#	%
OPTIC NERVE	# EX	3		0		3		1	
BONE, FEMUR	# EX	3		2		3		1	
Hypoplasia, epiphyseal cartilage		0	0.0	0	0.0	3	100.0	1	100.0
BONE MARROW, FEMORAL	# EX	3		2		3		1	
Depletion		0	0.0	0	0.0	3	100.0	0	0.0
BONE, STERNUM	# EX	3		2		3		1	
BONE MARROW, STERNUM	# EX	3		2		3		1	
Depletion		0	0.0	0	0.0	2	66.7	0	0.0
LYMPH NODE, MEDIASTINAL	# EX	2		0		0		0	
Sinus erythrocytosis		2	100.0	0	0.0	0	0.0	0	0.0
LYMPH NODE, DEEP CERVICAL	# EX	0		0		2		0	
Sinus erythrocytosis		0	0.0	0	0.0	2	100.0	0	0.0
Hyperplasia, lymphoid		0	0.0	0	0.0	1	50.0	0	0.0

Incidence Calculated by No. of Tissues Scored
(1) - 0 ug/kg body weight
(2) - 5 ug/kg body weight
(3) - 10 ug/kg body weight
(4) - 30 ug/kg body weight

LABCAT HP4.33

19-JAN-2001

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PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

PROJECT SUMMARY

STUDY ID : 1209 SN2 — STUDY NUMBER: 1209SN2
FATE: ALL
DAYS ON TEST: ALL — SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1 (1)		5 (2)		2 (3)		3 (4)	
NUMBER OF ANIMALS:		3		2		3		3	
		#	%	#	%	#	%	#	%
BRAIN, FORE	# EX	3		0		3		1	
Hemorrhage, acute, perivascular		0	0.0	0	0.0	1	33.3	0	0.0
SPINAL CORD, CERVICAL	# EX	3		0		3		1	
Hemorrhage, acute, perivascular		0	0.0	0	0.0	2	66.7	0	0.0
BRAIN, MID	# EX	3		0		3		1	
SPINAL CORD, THORACIC	# EX	3		0		3		1	
BRAIN, HIND	# EX	3		0		3		1	
HEART	# EX	3		2		3		1	
Inflammation, chronic, artery, auricle		1	33.3	0	0.0	0	0.0	0	0.0
Hyperplasia, serosa, focal		1	33.3	0	0.0	0	0.0	0	0.0
TRACHEA	# EX	3		0		3		1	
ESOPHAGUS	# EX	3		0		3		1	
AORTA	# EX	3		0		3		1	
LYMPH NODE, BRONCHIAL	# EX	3		0		3		1	
Sinus erythrocytosis		3	100.0	0	0.0	0	0.0	1	100.0
Depletion, lymphoid		0	0.0	0	0.0	0	0.0	1	100.0
LUNG	# EX	3		0		3		1	
Inflammation, subacute, focal		1	33.3	0	0.0	1	33.3	1	100.0
Inflammation, chronic, perivascular		2	66.7	0	0.0	1	33.3	0	0.0
Hemorrhage, acute, focal		1	33.3	0	0.0	0	0.0	0	0.0
Edema		1	33.3	0	0.0	0	0.0	0	0.0

Incidence Calculated by No. of Tissues Scored
(1) - 0 ug/kg body weight
(2) - 5 ug/kg body weight
(3) - 10 ug/kg body weight
(4) - 30 ug/kg body weight

LABCAT HP4.33 — 19-JAN-2001

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PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

PROJECT SUMMARY

STUDY ID : 1209 SN2
STUDY NUMBER: 1209SN2
FATE: ALL
DAYS ON TEST: ALL
SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1 (1)		5 (2)		2 (3)		3 (4)	
NUMBER OF ANIMALS:		3		2		3		3	
		#	%	#	%	#	%	#	%
KIDNEY	# EX	3		2		3		1	
Mineralization, medulla		3	100.0	2	100.0	3	100.0	1	100.0
Basophilic tubules		0	0.0	1	50.0	0	0.0	0	0.0
Dilatation, tubules		0	0.0	1	50.0	3	100.0	1	100.0
Mineralization, cortex		0	0.0	1	50.0	3	100.0	1	100.0
Basophilic tubules, diffuse		0	0.0	1	50.0	3	100.0	1	100.0
Congestion		0	0.0	0	0.0	0	0.0	1	100.0
Inflammation, chronic		0	0.0	1	50.0	1	33.3	0	0.0
SMALL INTESTINE, DUODENUM	# EX	3		0		3		1	
Dilatation, mucosal gland		0	0.0	0	0.0	2	66.7	0	0.0
Congestion		0	0.0	0	0.0	0	0.0	1	100.0
SPLEEN	# EX	3		2		3		1	
Mineralization, artery		0	0.0	0	0.0	2	66.7	0	0.0
PANCREAS	# EX	3		0		3		1	
LYMPH NODE, MESENTERIC	# EX	3		0		3		1	
Sinus erythrocytosis		3	100.0	0	0.0	1	33.3	1	100.0
Depletion, lymphoid		0	0.0	0	0.0	0	0.0	1	100.0
LIVER	# EX	3		0		3		1	
Inflammation, chronic, periportal		3	100.0	0	0.0	3	100.0	1	100.0
Inflammation, chronic, focal		3	100.0	0	0.0	3	100.0	0	0.0
GALLBLADDER	# EX	3		0		3		1	
Accumulation, lymphocyte		2	66.7	0	0.0	1	33.3	0	0.0
LARGE INTESTINE, RECTUM	# EX	3		0		3		1	
Dilatation, crypt glands		1	33.3	0	0.0	1	33.3	1	100.0

Incidence Calculated by No. of Tissues Scored
(1) - 0 ug/kg body weight
(2) - 5 ug/kg body weight
(3) - 10 ug/kg body weight
(4) - 30 ug/kg body weight

LABCAT HP4.33
19-JAN-2001

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

PROJECT SUMMARY

STUDY ID : 1209 SN2 STUDY NUMBER: 1209SN2
FATE: ALL
DAYS ON TEST: ALL SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1 (1)		5 (2)		2 (3)		3 (4)	
NUMBER OF ANIMALS:		3		2		3		3	
		#	%	#	%	#	%	#	%
LARGE INTESTINE, RECTUM	# EX	3		0		3		1	
Congestion		0	0.0	0	0.0	1	33.3	1	100.0
ADRENAL GLAND	# EX	3		2		3		1	
Mineralization, cortex, focal		0	0.0	0	0.0	1	33.3	0	0.0
Vacuolation, cortex		0	0.0	0	0.0	0	0.0	1	100.0
PERIPHERAL NERVE, SCIATIC	# EX	3		0		3		1	
SALIVARY GLAND	# EX	3		2		3		1	
Inflammation, chronic		1	33.3	0	0.0	0	0.0	0	0.0
TONGUE	# EX	3		0		3		1	
Inflammation, chronic, perivascular		3	100.0	0	0.0	3	100.0	0	0.0
Inflammation, subacute, focal		0	0.0	0	0.0	0	0.0	1	100.0
Erosion, focal		0	0.0	0	0.0	0	0.0	1	100.0
LYMPH NODE, MANDIBULAR	# EX	3		0		3		1	
Tattoo pigment		0	0.0	0	0.0	1	33.3	1	100.0
Granulopoiesis		1	33.3	0	0.0	0	0.0	0	0.0
SKIN, ELBOW	# EX	3		2		3		1	
Inflammation, subacute, dermis		1	33.3	1	50.0	0	0.0	0	0.0
SMALL INTESTINE, JEJUNUM	# EX	3		0		3		1	
Congestion		0	0.0	0	0.0	0	0.0	1	100.0
LARGE INTESTINE, COLON	# EX	3		0		3		1	
Dilatation, crypt glands		0	0.0	0	0.0	0	0.0	1	100.0
Congestion		0	0.0	0	0.0	0	0.0	1	100.0

Incidence Calculated by No. of Tissues Scored
(1) - 0 ug/kg body weight
(2) - 5 ug/kg body weight
(3) - 10 ug/kg body weight
(4) - 30 ug/kg body weight

LABCAT HP4.33 19-JAN-2001

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PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

PROJECT SUMMARY

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2

SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1 (1)		5 (2)		2 (3)		3 (4)	
NUMBER OF ANIMALS:		3		2		3		3	
		#	%	#	%	#	%	#	%
TONSIL	# EX	3		0		3		1	
Mineralization, focal		2	66.7	0	0.0	3	100.0	1	100.0
Inflammation, subacute		3	100.0	0	0.0	3	100.0	1	100.0
Hemorrhage		1	33.3	0	0.0	1	33.3	1	100.0
SKIN,DORSAL THORAX	# EX	3		2		3		1	
Inflammation, chronic, hair follicle		0	0.0	1	50.0	0	0.0	0	0.0
SMALL INTESTINE,ILEUM	# EX	3		0		3		1	
Congestion		0	0.0	0	0.0	0	0.0	1	100.0
THYMUS	# EX	3		2		3		1	
Atrophy		0	0.0	2	100.0	3	100.0	1	100.0
Hemorrhage, serosal		0	0.0	1	50.0	0	0.0	0	0.0
SKELETAL MUSCLE	# EX	3		2		3		1	
Atrophy		0	0.0	0	0.0	3	100.0	1	100.0
Inflammation, chronic, focal		1	33.3	0	0.0	0	0.0	0	0.0
Degeneration		0	0.0	0	0.0	0	0.0	1	100.0
Inflammation, subacute		0	0.0	0	0.0	0	0.0	1	100.0
SKIN	# EX	3		1		3		1	
MAMMARY GLAND	# EX	1		1		2		1	
THYROID GLAND	# EX	3		2		3		1	
Hypertrophy/hyperplasia, parafollicular cell		0	0.0	1	50.0	2	66.7	1	100.0
PARATHYROID GLAND	# EX	3		2		3		1	
Cyst		2	66.7	0	0.0	1	33.3	0	0.0
Hypertrophy		0	0.0	0	0.0	3	100.0	0	0.0

Incidence Calculated by No. of Tissues Scored
(1) - 0 ug/kg body weight
(2) - 5 ug/kg body weight
(3) - 10 ug/kg body weight
(4) - 30 ug/kg body weight

LABCAT HP4.33

19-JAN-2001

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PATHOLOGY ASSOCIATES
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1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

PROJECT SUMMARY

STUDY ID : 1209 SN2 — STUDY NUMBER: 1209SN2
FATE: ALL
DAYS ON TEST: ALL — SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1 (1)		5 (2)		2 (3)		3 (4)	
NUMBER OF ANIMALS:		3		2		3		3	
		#	%	#	%	#	%	#	%
PITUITARY GLAND	# EX	3		0		3		1	
Cyst		0	0.0	0	0.0	0	0.0	1	100.0
URETER	# EX	3		0		3		1	
STOMACH	# EX	3		2		3		1	
Mineralization, focal		1	33.3	0	0.0	0	0.0	0	0.0
Accumulation, lymphocyte		1	33.3	2	100.0	1	33.3	1	100.0
Mineralization, mid-mucosal, pyloric		0	0.0	1	50.0	2	66.7	1	100.0
Congestion		0	0.0	0	0.0	0	0.0	1	100.0
LARGE INTESTINE, CECUM	# EX	3		0		3		1	
Dilatation, crypt gland		0	0.0	0	0.0	1	33.3	1	100.0
Congestion		1	33.3	0	0.0	0	0.0	1	100.0
URINARY BLADDER	# EX	3		0		3		1	
Accumulation, lymphocyte		1	33.3	0	0.0	0	0.0	0	0.0
Inflammation, subacute		1	33.3	0	0.0	0	0.0	0	0.0
Inflammation, chronic, perivascular		1	33.3	0	0.0	0	0.0	0	0.0
OVARY	# EX	3		2		3		1	
FALLOPIAN TUBE	# EX	2		0		3		1	
UTERUS	# EX	3		2		3		1	
Atrophy		0	0.0	0	0.0	3	100.0	1	100.0
VAGINA	# EX	3		0		3		1	
CERVIX	# EX	3		0		3		1	

Incidence Calculated by No. of Tissues Scored
(1) - 0 ug/kg body weight
(2) - 5 ug/kg body weight
(3) - 10 ug/kg body weight
(4) - 30 ug/kg body weight

LABCAT HP4.33 — 19-JAN-2001

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

PROJECT SUMMARY

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2

SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1 (1)		5 (2)		2 (3)		3 (4)	
NUMBER OF ANIMALS:		3		2		3		3	
		#	%	#	%	#	%	#	%
EYE	# EX	3		0		3		1	
OPTIC NERVE	# EX	3		0		3		1	
BONE, FEMUR	# EX	3		2		3		1	
Hypoplasia, epiphyseal cartilage		0	0.0	0	0.0	3	100.0	1	100.0
BONE MARROW, FEMORAL	# EX	3		2		3		1	
Depletion		0	0.0	0	0.0	3	100.0	1	100.0
BONE, STERNUM	# EX	3		2		3		1	
BONE MARROW, STERNUM	# EX	3		2		3		1	
Depletion		0	0.0	0	0.0	2	66.7	1	100.0
LYMPH NODE, MEDIASTINAL	# EX	2		0		1		0	
Sinus erythrocytosis		2	100.0	0	0.0	1	100.0	0	0.0
MESENTERY	# EX	0		0		1		0	
Cyst, blood		0	0.0	0	0.0	1	100.0	0	0.0

Incidence Calculated by No. of Tissues Scored
(1) - 0 ug/kg body weight
(2) - 5 ug/kg body weight
(3) - 10 ug/kg body weight
(4) - 30 ug/kg body weight

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19-JAN-2001

SECTION III

SEVERITY SUMMARY

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PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

SEVERITY SUMMARY

STUDY NUMBER: 1209SN2

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

SEX: MALE

GROUP:		1 (1)	5 (2)	2 (3)	3 (4)
NUMBER OF ANIMALS:		3	2	3	3
		# SEV	# SEV	# SEV	# SEV
BRAIN, FORE	# EX	3	0	3	1
Hemorrhage, acute, perivascular		0 0.00	0 0.00	1 0.33	0 0.00
SPINAL CORD, CERVICAL	# EX	3	0	3	1
BRAIN, MID	# EX	3	0	3	1
Mineralization, meninges		0 0.00	0 0.00	0 0.00	1 1.00
SPINAL CORD, THORACIC	# EX	3	0	3	1
BRAIN, HIND	# EX	3	0	3	1
HEART	# EX	3	2	3	1
Mineralization, aortic base		0 0.00	0 0.00	1 0.67	1 3.00
TRACHEA	# EX	3	0	3	1
Inflammation, subacute		1 0.33	0 0.00	0 0.00	0 0.00
Mineralization, focal		0 0.00	0 0.00	0 0.00	1 1.00
ESOPHAGUS	# EX	3	0	3	1
AORTA	# EX	3	0	3	1
LYMPH NODE, BRONCHIAL	# EX	3	0	3	1
Sinus erythrocytosis		2 1.00	0 0.00	0 0.00	0 0.00
Depletion, lymphoid		0 0.00	0 0.00	0 0.00	1 2.00
LUNG	# EX	3	0	3	1
Inflammation, subacute, focal		2 1.33	0 0.00	1 0.33	0 0.00
Inflammation, chronic, perivascular		2 0.67	0 0.00	1 0.33	1 1.00
Hemorrhage, acute, focal		1 0.33	0 0.00	0 0.00	0 0.00
Inflammation, granulomatous, focal		0 0.00	0 0.00	1 0.33	0 0.00

Severity Calculated by No. of Tissues Scored
(1) - 0 ug/kg body weight
(2) - 5 ug/kg body weight
(3) - 10 ug/kg body weight
(4) - 30 ug/kg body weight

19-JAN-2001

LABCAT HP4.33

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PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

SEVERITY SUMMARY

STUDY NUMBER: 1209SN2

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

SEX: MALE

		1	5	2	3
GROUP:		(1)	(2)	(3)	(4)
NUMBER OF ANIMALS:		3	2	3	3
		# SEV	# SEV	# SEV	# SEV
KIDNEY	# EX	3	2	3	1
Mineralization, medulla		3 1.00	2 1.00	3 1.33	1 2.00
Basophilic tubules		1 0.33	0 0.00	0 0.00	0 0.00
Dilatation, tubules		0 0.00	1 1.00	3 3.00	1 3.00
Mineralization, cortex		0 0.00	1 0.50	3 2.00	1 3.00
Basophilic tubules, diffuse		0 0.00	1 1.00	3 3.00	1 3.00
Congestion		0 0.00	0 0.00	3 2.00	0 0.00
Dilatation, pelvis		0 0.00	0 0.00	1 0.67	0 0.00
Inflammation, chronic		0 0.00	2 1.50	0 0.00	1 2.00
SMALL INTESTINE, DUODENUM	# EX	3	0	3	1
Dilatation, mucosal gland		1 0.33	0 0.00	1 0.33	1 2.00
Congestion		0 0.00	0 0.00	0 0.00	1 2.00
SPLEEN	# EX	3	2	3	1
Mineralization, artery		0 0.00	0 0.00	0 0.00	1 2.00
PANCREAS	# EX	3	0	3	1
LYMPH NODE, MESENTERIC	# EX	3	0	3	1
Sinus erythrocytosis		3 1.00	0 0.00	1 0.33	0 0.00
Depletion, lymphoid		0 0.00	0 0.00	0 0.00	1 2.00
LIVER	# EX	3	0	3	1
Inflammation, chronic, periportal		2 0.67	0 0.00	2 0.67	1 1.00
Inflammation, granulomatous, focal		0 0.00	0 0.00	1 0.33	0 0.00
Inflammation, chronic, focal		0 0.00	0 0.00	1 0.33	0 0.00
GALLBLADDER	# EX	3	0	3	1
Accumulation, lymphocyte		3 1.00	0 0.00	0 0.00	0 0.00

Severity Calculated by No. of Tissues Scored
(1) - 0 ug/kg body weight
(2) - 5 ug/kg body weight
(3) - 10 ug/kg body weight
(4) - 30 ug/kg body weight

19-JAN-2001

LABCAT HP4.33

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PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

SEVERITY SUMMARY

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2

SEX: MALE

GROUP:		1 (1)	5 (2)	2 (3)	3 (4)
NUMBER OF ANIMALS:		3	2	3	3
		# SEV	# SEV	# SEV	# SEV
LARGE INTESTINE, RECTUM	# EX	3	0	3	1
Dilatation, crypt glands		0 0.00	0 0.00	1 0.67	0 0.00
Congestion		0 0.00	0 0.00	1 0.67	1 2.00
ADRENAL GLAND	# EX	3	2	3	1
PERIPHERAL NERVE, SCIATIC	# EX	3	0	3	1
SALIVARY GLAND	# EX	3	2	3	1
Necrosis, focal, parotid		0 0.00	0 0.00	1 0.67	0 0.00
Mineralization, focal, parotid		0 0.00	0 0.00	1 0.67	0 0.00
TONGUE	# EX	3	0	3	1
Inflammation, chronic, perivascular		1 0.67	0 0.00	1 0.33	0 0.00
Inflammation, subacute, focal		0 0.00	0 0.00	1 0.67	0 0.00
Erosion, focal		0 0.00	0 0.00	1 0.67	0 0.00
LYMPH NODE, MANDIBULAR	# EX	3	0	3	1
Sinus erythrocytosis		2 0.67	0 0.00	1 0.67	1 1.00
SKIN, ELBOW	# EX	3	2	3	1
Inflammation, subacute, dermis		0 0.00	1 0.50	0 0.00	0 0.00
SMALL INTESTINE, JEJUNUM	# EX	3	0	3	1
Dilatation, crypt glands		0 0.00	0 0.00	0 0.00	1 1.00
Congestion		0 0.00	0 0.00	0 0.00	1 2.00
LARGE INTESTINE, COLON	# EX	3	0	3	1
Dilatation, crypt glands		0 0.00	0 0.00	1 0.67	1 2.00
Congestion		0 0.00	0 0.00	1 0.33	1 2.00

Severity Calculated by No. of Tissues Scored
(1) - 0 ug/kg body weight
(2) - 5 ug/kg body weight
(3) - 10 ug/kg body weight
(4) - 30 ug/kg body weight

LABCAT HP4.33

19-JAN-2001

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PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

SEVERITY SUMMARY

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
SEX: MALE

GROUP:		1 (1)	5 (2)	2 (3)	3 (4)
NUMBER OF ANIMALS:		3	2	3	3
		# SEV	# SEV	# SEV	# SEV
TONSIL	# EX	3	2	3	1
Mineralization, focal		3 1.00	2 1.00	3 1.00	1 2.00
Inflammation, subacute		3 1.00	2 1.00	3 1.00	1 1.00
Hemorrhage		3 1.33	2 1.50	3 1.33	1 2.00
SKIN, DORSAL THORAX	# EX	3	2	3	1
SMALL INTESTINE, ILEUM	# EX	3	1	3	1
Congestion		0 0.00	0 0.00	0 0.00	1 2.00
THYMUS	# EX	3	2	3	1
Atrophy		0 0.00	0 0.00	3 3.33	1 4.00
SKELETAL MUSCLE	# EX	3	2	3	1
Atrophy		0 0.00	0 0.00	3 2.00	1 3.00
SKIN	# EX	3	0	3	1
Abscess		0 0.00	0 0.00	1 1.33	0 0.00
Ulceration		0 0.00	0 0.00	1 1.33	1 4.00
MAMMARY GLAND	# EX	3	0	0	1
THYROID GLAND	# EX	3	2	3	1
Hypertrophy/hyperplasia, parafollicular cell		0 0.00	0 0.00	3 1.67	1 3.00
PARATHYROID GLAND	# EX	3	2	3	1
Cyst		1 0.67	1 1.00	1 0.67	1 1.00
Hypertrophy		0 0.00	0 0.00	0 0.00	1 2.00
PITUITARY GLAND	# EX	3	0	3	1
Cyst		0 0.00	0 0.00	2 1.33	0 0.00

Severity Calculated by No. of Tissues Scored
(1) - 0 ug/kg body weight
(2) - 5 ug/kg body weight
(3) - 10 ug/kg body weight
(4) - 30 ug/kg body weight

LABCAT HP4.33

19-JAN-2001

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

SEVERITY SUMMARY

STUDY ID : 1209 SN2 — STUDY NUMBER: 1209SN2
FATE: ALL
DAYS ON TEST: ALL — SEX: MALE

GROUP:		1 (1)	5 (2)	2 (3)	3 (4)
NUMBER OF ANIMALS:		3	2	3	3
		# SEV	# SEV	# SEV	# SEV
URETER	# EX	3	0	3	1
STOMACH	# EX	3	2	3	1
Mineralization, focal		1 0.33	0 0.00	0 0.00	0 0.00
Accumulation, lymphocyte		1 1.00	2 1.50	1 0.67	1 1.00
Mineralization, mid-mucosal, pyloric		0 0.00	1 0.50	1 1.00	1 3.00
LARGE INTESTINE, CECUM	# EX	3	1	3	1
Dilatation, crypt gland		0 0.00	0 0.00	1 0.33	0 0.00
Congestion		0 0.00	0 0.00	1 0.33	0 0.00
URINARY BLADDER	# EX	3	0	3	1
TESTES	# EX	3	2	3	1
EPIDIDYMIS	# EX	3	0	3	1
Oligospermia		2 2.33	0 0.00	3 4.00	1 4.00
PROSTATE	# EX	3	2	3	1
EYE	# EX	3	0	3	1
OPTIC NERVE	# EX	3	0	3	1
BONE, FEMUR	# EX	3	2	3	1
Hypoplasia, epiphyseal cartilage		0 0.00	0 0.00	3 2.00	1 2.00
BONE MARROW, FEMORAL	# EX	3	2	3	1
Depletion		0 0.00	0 0.00	3 2.67	0 0.00
BONE, STERNUM	# EX	3	2	3	1

Severity Calculated by No. of Tissues Scored
(1) - 0 ug/kg body weight
(2) - 5 ug/kg body weight
(3) - 10 ug/kg body weight
(4) - 30 ug/kg body weight

LABCAT HP4.33 — 19-JAN-2001

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PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

SEVERITY SUMMARY

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2

SEX: MALE

GROUP:		1 (1)		5 (2)		2 (3)		3 (4)	
NUMBER OF ANIMALS:		3		2		3		3	
		#	SEV	#	SEV	#	SEV	#	SEV
BONE MARROW, STERNUM	# EX	3		2		3		1	
Depletion		0	0.00	0	0.00	2	1.00	0	0.00
LYMPH NODE, MEDIASTINAL	# EX	2		0		0		0	
Sinus erythrocytosis		2	3.00	0	0.00	0	0.00	0	0.00
LYMPH NODE, DEEP CERVICAL	# EX	0		0		2		0	
Sinus erythrocytosis		0	0.00	0	0.00	2	2.50	0	0.00
Hyperplasia, lymphoid		0	0.00	0	0.00	1	1.00	0	0.00

Severity Calculated by No. of Tissues Scored
(1) - 0 ug/kg body weight
(2) - 5 ug/kg body weight
(3) - 10 ug/kg body weight
(4) - 30 ug/kg body weight

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19-JAN-2001

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

SEVERITY SUMMARY

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2

SEX: FEMALE

GROUP:		1 (1)	5 (2)	2 (3)	3 (4)
NUMBER OF ANIMALS:		3	2	3	3
		# SEV	# SEV	# SEV	# SEV
BRAIN, FORE	# EX	3	0	3	1
Hemorrhage, acute, perivascular		0 0.00	0 0.00	1 0.33	0 0.00
SPINAL CORD, CERVICAL	# EX	3	0	3	1
Hemorrhage, acute, perivascular		0 0.00	0 0.00	2 0.67	0 0.00
BRAIN, MID	# EX	3	0	3	1
SPINAL CORD, THORACIC	# EX	3	0	3	1
BRAIN, HIND	# EX	3	0	3	1
HEART	# EX	3	2	3	1
Inflammation, chronic, artery, auricle		1 0.67	0 0.00	0 0.00	0 0.00
Hyperplasia, serosa, focal		1 0.67	0 0.00	0 0.00	0 0.00
TRACHEA	# EX	3	0	3	1
ESOPHAGUS	# EX	3	0	3	1
AORTA	# EX	3	0	3	1
LYMPH NODE, BRONCHIAL	# EX	3	0	3	1
Sinus erythrocytosis		3 1.67	0 0.00	0 0.00	1 2.00
Depletion, lymphoid		0 0.00	0 0.00	0 0.00	1 2.00
LUNG	# EX	3	0	3	1
Inflammation, subacute, focal		1 0.67	0 0.00	1 0.33	1 3.00
Inflammation, chronic, perivascular		2 0.67	0 0.00	1 0.33	0 0.00
Hemorrhage, acute, focal		1 1.00	0 0.00	0 0.00	0 0.00
Edema		1 1.00	0 0.00	0 0.00	0 0.00

Severity Calculated by No. of Tissues Scored
(1) - 0 ug/kg body weight
(2) - 5 ug/kg body weight
(3) - 10 ug/kg body weight
(4) - 30 ug/kg body weight

19-JAN-2001

LABCAT HP4.33

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PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

SEVERITY SUMMARY

STUDY ID : 1209 SN2
STUDY NUMBER: 1209SN2
FATE: ALL
DAYS ON TEST: ALL
SEX: FEMALE

GROUP:		1 (1)		5 (2)		2 (3)		3 (4)	
NUMBER OF ANIMALS:		3		2		3		3	
		#	SEV	#	SEV	#	SEV	#	SEV
KIDNEY	# EX	3		2		3		1	
Mineralization, medulla		3	1.33	2	1.50	3	1.00	1	2.00
Basophilic tubules		0	0.00	1	0.50	0	0.00	0	0.00
Dilatation, tubules		0	0.00	1	0.50	3	3.33	1	3.00
Mineralization, cortex		0	0.00	1	0.50	3	2.00	1	3.00
Basophilic tubules, diffuse		0	0.00	1	0.50	3	3.00	1	3.00
Congestion		0	0.00	0	0.00	0	0.00	1	2.00
Inflammation, chronic		0	0.00	1	1.00	1	0.33	0	0.00
SMALL INTESTINE,DUODENUM	# EX	3		0		3		1	
Dilatation, mucosal gland		0	0.00	0	0.00	2	1.33	0	0.00
Congestion		0	0.00	0	0.00	0	0.00	1	2.00
SPLEEN	# EX	3		2		3		1	
Mineralization, artery		0	0.00	0	0.00	2	1.00	0	0.00
PANCREAS	# EX	3		0		3		1	
LYMPH NODE,MESENTERIC	# EX	3		0		3		1	
Sinus erythrocytosis		3	1.00	0	0.00	1	0.33	1	3.00
Depletion, lymphoid		0	0.00	0	0.00	0	0.00	1	2.00
LIVER	# EX	3		0		3		1	
Inflammation, chronic, periportal		3	1.00	0	0.00	3	1.00	1	2.00
Inflammation, chronic, focal		3	1.00	0	0.00	3	1.00	0	0.00
GALLBLADDER	# EX	3		0		3		1	
Accumulation, lymphocyte		2	0.67	0	0.00	1	0.33	0	0.00
LARGE INTESTINE,RECTUM	# EX	3		0		3		1	
Dilatation, crypt glands		1	0.33	0	0.00	1	0.33	1	2.00
Congestion		0	0.00	0	0.00	1	0.33	1	2.00

Severity Calculated by No. of Tissues Scored
(1) - 0 ug/kg body weight
(2) - 5 ug/kg body weight
(3) - 10 ug/kg body weight
(4) - 30 ug/kg body weight

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19-JAN-2001

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PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

SEVERITY SUMMARY

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2

SEX: FEMALE

GROUP:		1 (1)	5 (2)	2 (3)	3 (4)
NUMBER OF ANIMALS:		3	2	3	3
		# SEV	# SEV	# SEV	# SEV
ADRENAL GLAND	# EX	3	2	3	1
Mineralization, cortex, focal		0 0.00	0 0.00	1 0.67	0 0.00
Vacuolation, cortex		0 0.00	0 0.00	0 0.00	1 2.00
PERIPHERAL NERVE,SCIATIC	# EX	3	0	3	1
SALIVARY GLAND	# EX	3	2	3	1
Inflammation, chronic		1 0.33	0 0.00	0 0.00	0 0.00
TONGUE	# EX	3	0	3	1
Inflammation, chronic, perivascular		3 1.33	0 0.00	3 1.00	0 0.00
Inflammation, subacute, focal		0 0.00	0 0.00	0 0.00	1 2.00
Erosion, focal		0 0.00	0 0.00	0 0.00	1 2.00
LYMPH NODE,MANDIBULAR	# EX	3	0	3	1
Granulopoiesis		1 0.33	0 0.00	0 0.00	0 0.00
SKIN,ELBOW	# EX	3	2	3	1
Inflammation, subacute, dermis		1 0.67	1 0.50	0 0.00	0 0.00
SMALL INTESTINE,JEJUNUM	# EX	3	0	3	1
Congestion		0 0.00	0 0.00	0 0.00	1 1.00
LARGE INTESTINE,COLON	# EX	3	0	3	1
Dilatation, crypt glands		0 0.00	0 0.00	0 0.00	1 2.00
Congestion		0 0.00	0 0.00	0 0.00	1 2.00
TONSIL	# EX	3	0	3	1
Mineralization, focal		2 0.67	0 0.00	3 1.00	1 1.00
Inflammation, subacute		3 1.00	0 0.00	3 1.33	1 1.00
Hemorrhage		1 0.67	0 0.00	1 0.33	1 2.00

Severity Calculated by No. of Tissues Scored
(1) - 0 ug/kg body weight
(2) - 5 ug/kg body weight
(3) - 10 ug/kg body weight
(4) - 30 ug/kg body weight

LABCAT HP4.33

19-JAN-2001

DRAFT

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

SEVERITY SUMMARY

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2

SEX: FEMALE

GROUP:		1 (1)	5 (2)	2 (3)	3 (4)
NUMBER OF ANIMALS:		3	2	3	3
		# SEV	# SEV	# SEV	# SEV
SKIN,DORSAL THORAX	# EX	3	2	3	1
Inflammation, chronic, hair follicle		0 0.00	1 0.50	0 0.00	0 0.00
SMALL INTESTINE,ILEUM	# EX	3	0	3	1
Congestion		0 0.00	0 0.00	0 0.00	1 2.00
THYMUS	# EX	3	2	3	1
Atrophy		0 0.00	2 1.50	3 2.67	1 4.00
Hemorrhage, serosal		0 0.00	1 1.50	0 0.00	0 0.00
SKELETAL MUSCLE	# EX	3	2	3	1
Atrophy		0 0.00	0 0.00	3 2.00	1 3.00
Inflammation, chronic, focal		1 0.33	0 0.00	0 0.00	0 0.00
Degeneration		0 0.00	0 0.00	0 0.00	1 3.00
Inflammation, subacute		0 0.00	0 0.00	0 0.00	1 2.00
SKIN	# EX	3	1	3	1
MAMMARY GLAND	# EX	1	1	2	1
THYROID GLAND	# EX	3	2	3	1
Hypertrophy/hyperplasia, parafollicular cell		0 0.00	1 0.50	2 0.67	1 3.00
PARATHYROID GLAND	# EX	3	2	3	1
Cyst		2 0.67	0 0.00	1 0.67	0 0.00
Hypertrophy		0 0.00	0 0.00	3 1.00	0 0.00
PITUITARY GLAND	# EX	3	0	3	1
Cyst		0 0.00	0 0.00	0 0.00	1 1.00
URETER	# EX	3	0	3	1

Severity Calculated by No. of Tissues Scored
(1) - 0 ug/kg body weight
(2) - 5 ug/kg body weight
(3) - 10 ug/kg body weight
(4) - 30 ug/kg body weight

LABCAT HP4.33

19-JAN-2001

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PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

SEVERITY SUMMARY

STUDY ID : 1209 SN2 — STUDY NUMBER: 1209SN2
FATE: ALL
DAYS ON TEST: ALL — SEX: FEMALE

GROUP:		1 (1)	5 (2)	2 (3)	3 (4)
NUMBER OF ANIMALS:		3	2	3	3
		# SEV	# SEV	# SEV	# SEV
STOMACH	# EX	3	2	3	1
Mineralization, focal		1 0.67	0 0.00	0 0.00	0 0.00
Accumulation, lymphocyte		1 0.67	2 2.00	1 0.67	1 1.00
Mineralization, mid-mucosal, pyloric		0 0.00	1 1.00	2 1.33	1 4.00
Congestion		0 0.00	0 0.00	0 0.00	1 2.00
LARGE INTESTINE,CECUM	# EX	3	0	3	1
Dilatation, crypt gland		0 0.00	0 0.00	1 0.33	1 1.00
Congestion		1 0.33	0 0.00	0 0.00	1 2.00
URINARY BLADDER	# EX	3	0	3	1
Accumulation, lymphocyte		1 0.67	0 0.00	0 0.00	0 0.00
Inflammation, subacute		1 0.67	0 0.00	0 0.00	0 0.00
Inflammation, chronic, perivascular		1 0.67	0 0.00	0 0.00	0 0.00
OVARY	# EX	3	2	3	1
FALLOPIAN TUBE	# EX	2	0	3	1
UTERUS	# EX	3	2	3	1
Atrophy		0 0.00	0 0.00	3 2.33	1 3.00
VAGINA	# EX	3	0	3	1
CERVIX	# EX	3	0	3	1
EYE	# EX	3	0	3	1
OPTIC NERVE	# EX	3	0	3	1
BONE,FEMUR	# EX	3	2	3	1
Hypoplasia, epiphyseal cartilage		0 0.00	0 0.00	3 2.00	1 2.00

Severity Calculated by No. of Tissues Scored
(1) - 0 ug/kg body weight
(2) - 5 ug/kg body weight
(3) - 10 ug/kg body weight
(4) - 30 ug/kg body weight

LABCAT HP4.33 — 19-JAN-2001

DRAFT

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

SEVERITY SUMMARY

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2

SEX: FEMALE

GROUP:		1 (1)		5 (2)		2 (3)		3 (4)	
NUMBER OF ANIMALS:		3		2		3		3	
		#	SEV	#	SEV	#	SEV	#	SEV
BONE MARROW, FEMORAL	# EX	3		2		3		1	
Depletion		0	0.00	0	0.00	3	2.33	1	3.00
BONE, STERNUM	# EX	3		2		3		1	
BONE MARROW, STERNUM	# EX	3		2		3		1	
Depletion		0	0.00	0	0.00	2	0.67	1	3.00
LYMPH NODE, MEDIASTINAL	# EX	2		0		1		0	
Sinus erythrocytosis		2	3.00	0	0.00	1	3.00	0	0.00
MESENTERY	# EX	0		0		1		0	
Cyst, blood		0	0.00	0	0.00	1	2.00	0	0.00

Severity Calculated by No. of Tissues Scored
(1) - 0 ug/kg body weight
(2) - 5 ug/kg body weight
(3) - 10 ug/kg body weight
(4) - 30 ug/kg body weight

LABCAT HP4.33

19-JAN-2001

SECTION IV

TABULATED ANIMAL DATA

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PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 1: 0 ug/kg body weight
SEX: MALE

ANIMAL ID:	1252	1256	1263
BRAIN, FORE	N	N	N
SPINAL CORD, CERVICAL	N	N	N
BRAIN, MID	N	N	N
SPINAL CORD, THORACIC	N	N	N
BRAIN, HIND	N	N	N
HEART	N	N	N
TRACHEA	-	N	N
Inflammation, subacute	1	-	-
ESOPHAGUS	N	N	N
AORTA	N	N	N
LYMPH NODE, BRONCHIAL	-	N	-
Sinus erythrocytosis	2	-	1
LUNG	-	-	-
Inflammation, subacute, focal	2	2	-
Inflammation, chronic, perivascular	-	1	1
Hemorrhage, acute, focal	-	-	1
KIDNEY	-	-	-
Mineralization, medulla	1	1	1
Basophilic tubules	1	-	-
SMALL INTESTINE, DUODENUM	N	N	-
Dilatation, mucosal gland	-	-	1

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 1: 0 ug/kg body weight
SEX: MALE

ANIMAL ID:	1252	1256	1263
SPLEEN	N	N	N
PANCREAS	N	N	N
LYMPH NODE, MESENTERIC	-	-	-
Sinus erythrocytosis	1	1	1
LIVER	N	-	-
Inflammation, chronic, periportal	-	1	1
GALLBLADDER	-	-	-
Accumulation, lymphocyte	1	1	1
LARGE INTESTINE, RECTUM	N	N	N
ADRENAL GLAND	N	N	N
PERIPHERAL NERVE, SCIATIC	N	N	N
SALIVARY GLAND	N	N	N
TONGUE	N	N	-
Inflammation, chronic, perivascular	-	-	2
LYMPH NODE, MANDIBULAR	N	-	-
Sinus erythrocytosis	-	1	1
SKIN, ELBOW	N	N	N
SMALL INTESTINE, JEJUNUM	N	N	N
LARGE INTESTINE, COLON	N	N	N
TONSIL	-	-	-
Mineralization, focal	1	1	1

See Reports Code Table for Symbol Definitions

19-JAN-2001

LABCAT HP4.33

DRAFT

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 1: 0 ug/kg body weight
SEX: MALE

ANIMAL ID:	1252	1256	1263
Inflammation, subacute	1	1	1
Hemorrhage	1	2	1
SKIN,DORSAL THORAX	N	N	N
SMALL INTESTINE,ILEUM	N	N	N
THYMUS	N	N	N
SKELETAL MUSCLE	N	N	N
SKIN	N	N	N
MAMMARY GLAND	N	N	N
THYROID GLAND	N	N	N
PARATHYROID GLAND	N	N	-
Cyst	-	-	2
PITUITARY GLAND	N	N	N
URETER	N	N	N
STOMACH	-	N	-
Mineralization, focal	1	-	-
Accumulation, lymphocyte	-	-	3
LARGE INTESTINE,CECUM	N	N	N
URINARY BLADDER	N	N	N
TESTES	N	-	-
Sexual immaturity	-	P	P

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 1: 0 ug/kg body weight
SEX: MALE

ANIMAL ID:	1252	1256	1263
EPIDIDYMIS	N	-	-
Oligospermia	-	3	4
PROSTATE	-	-	-
Sexual immaturity	P	P	P
EYE	N	N	N
OPTIC NERVE	N	N	N
BONE, FEMUR	N	N	N
BONE MARROW, FEMORAL	N	N	N
BONE, STERNUM	N	N	N
BONE MARROW, STERNUM	N	N	N
Non-Protocol Tissues:			
LYMPH NODE, MEDIASTINAL	-	-	-
Sinus erythrocytosis	-	3	3

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 5: 5 ug/kg body weight
SEX: MALE

ANIMAL ID:	1258	1266
HEART	N	N
KIDNEY	-	-
Mineralization, medulla	1	1
Dilatation, tubules	-	2
Mineralization, cortex	-	1
Basophilic tubules, diffuse	-	2
Inflammation, chronic	1	2
SPLEEN	N	N
ADRENAL GLAND	N	N
SALIVARY GLAND	N	N
SKIN, ELBOW	-	N
Inflammation, subacute, dermis	1	-
TONSIL	-	-
Mineralization, focal	1	1
Inflammation, subacute	1	1
Hemorrhage	2	1
SKIN, DORSAL THORAX	N	N
SMALL INTESTINE, ILEUM	-	N
THYMUS	N	N
SKELETAL MUSCLE	N	N
THYROID GLAND	N	N
PARATHYROID GLAND	-	N
Cyst	2	-

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

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PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 5: 5 ug/kg body weight
SEX: MALE

ANIMAL ID:	1258	1266
STOMACH	-	-
Accumulation, lymphocyte	1	2
Mineralization, mid-mucosal, pyloric	-	1
LARGE INTESTINE, CECUM	-	N
TESTES	-	N
Sexual immaturity	P	-
PROSTATE	-	-
Sexual immaturity	P	P
BONE, FEMUR	N	N
BONE MARROW, FEMORAL	N	N
BONE, STERNUM	N	N
BONE MARROW, STERNUM	N	N

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

DRAFT

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 2: 10 ug/kg body weight
SEX: MALE

ANIMAL ID:	1257	1260	1262
BRAIN, FORE	-	N	N
Hemorrhage, acute, perivascular	1	-	-
SPINAL CORD, CERVICAL	N	N	N
BRAIN, MID	N	N	N
SPINAL CORD, THORACIC	N	N	N
BRAIN, HIND	N	N	N
HEART	N	N	-
Mineralization, aortic base	-	-	2
TRACHEA	N	N	N
ESOPHAGUS	N	N	N
AORTA	N	N	N
LYMPH NODE, BRONCHIAL	N	N	N
LUNG	-	-	-
Inflammation, subacute, focal	-	1	-
Inflammation, chronic, perivascular	-	-	1
Inflammation, granulomatous, focal	1	-	-
KIDNEY	-	-	-
Mineralization, medulla	1	1	2
Dilatation, tubules	3	3	3
Mineralization, cortex	2	2	2
Basophilic tubules, diffuse	3	3	3
Congestion	2	2	2
Dilatation, pelvis	-	-	2

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

DRAFT

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 2: 10 ug/kg body weight
SEX: MALE

ANIMAL ID:	1257	1260	1262
SMALL INTESTINE, DUODENUM	N	N	-
Dilatation, mucosal gland	-	-	1
SPLEEN	N	N	N
PANCREAS	N	N	N
LYMPH NODE, MESENTERIC	N	N	-
Sinus erythrocytosis	-	-	1
LIVER	-	-	-
Inflammation, chronic, periportal	1	-	1
Inflammation, granulomatous, focal	1	-	-
Inflammation, chronic, focal	-	1	-
GALLBLADDER	N	N	N
LARGE INTESTINE, RECTUM	N	N	-
Dilatation, crypt glands	-	-	2
Congestion	-	-	2
ADRENAL GLAND	N	N	N
PERIPHERAL NERVE, SCIATIC	N	N	N
SALIVARY GLAND	N	N	-
Necrosis, focal, parotid	-	-	2
Mineralization, focal, parotid	-	-	2
TONGUE	-	N	N
Inflammation, chronic, perivascular	1	-	-
Inflammation, subacute, focal	2	-	-
Erosion, focal	2	-	-
LYMPH NODE, MANDIBULAR	N	-	-

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

DRAFT

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 2: 10 ug/kg body weight
SEX: MALE

ANIMAL ID:	1257	1260	1262
Sinus erythrocytosis	-	-	2
Tattoo pigment	-	P	-
SKIN,ELBOW	N	N	N
SMALL INTESTINE,JEJUNUM	N	N	N
LARGE INTESTINE,COLON	N	N	-
Dilatation, crypt glands	-	-	2
Congestion	-	-	1
TONSIL	-	-	-
Mineralization, focal	1	1	1
Inflammation, subacute	1	1	1
Hemorrhage	1	2	1
SKIN,DORSAL THORAX	N	N	N
SMALL INTESTINE,ILEUM	N	N	N
THYMUS	-	-	-
Atrophy	3	3	4
SKELETAL MUSCLE	-	-	-
Atrophy	2	2	2
SKIN	N	N	-
Abscess	-	-	4
Bacteria	-	-	P
Ulceration	-	-	4
MAMMARY GLAND	U	U	U
THYROID GLAND	-	-	-
Hypertrophy/hyperplasia, parafollicular cell	1	1	3

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 2: 10 ug/kg body weight
SEX: MALE

ANIMAL ID:	1257	1260	1262
PARATHYROID GLAND	N	-	N
Cyst	-	2	-
PITUITARY GLAND	-	-	N
Cyst	2	2	-
URETER	N	N	N
STOMACH	-	N	-
Accumulation, lymphocyte	-	-	2
Mineralization, mid-mucosal, pyloric	3	-	-
LARGE INTESTINE, CECUM	N	N	-
Dilatation, crypt gland	-	-	1
Congestion	-	-	1
URINARY BLADDER	N	N	N
TESTES	-	-	-
Sexual immaturity	P	P	P
EPIDIDYMIS	-	-	-
Oligospermia	4	4	4
PROSTATE	-	-	-
Sexual immaturity	P	P	P
EYE	N	N	N
OPTIC NERVE	N	N	N
BONE, FEMUR	-	-	-
Hypoplasia, epiphyseal cartilage	2	2	2
BONE MARROW, FEMORAL	-	-	-

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

DRAFT

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 2: 10 ug/kg body weight
SEX: MALE

ANIMAL ID:	1257	1260	1262
Depletion	2	3	3
BONE, STERNUM	N	N	N
BONE MARROW, STERNUM	N	-	-
Depletion	-	1	2
Non-Protocol Tissues:			
LYMPH NODE, DEEP CERVICAL	-	-	-
Sinus erythrocytosis	-	3	2
Hyperplasia, lymphoid	-	-	2

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

DRAFT

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 3: 30 ug/kg body weight
SEX: MALE

ANIMAL ID:	1259	1261	1265
BRAIN, FORE	-	N	-
SPINAL CORD, CERVICAL	-	N	-
BRAIN, MID	-	-	-
Mineralization, meninges	-	1	-
SPINAL CORD, THORACIC	-	N	-
BRAIN, HIND	-	N	-
HEART	-	-	-
Mineralization, aortic base	-	3	-
TRACHEA	-	-	-
Mineralization, focal	-	1	-
ESOPHAGUS	-	N	-
AORTA	-	N	-
LYMPH NODE, BRONCHIAL	-	-	-
Depletion, lymphoid	-	2	-
LUNG	-	-	-
Inflammation, chronic, perivascular	-	1	-
KIDNEY	-	-	-
Mineralization, medulla	-	2	-
Dilatation, tubules	-	3	-
Mineralization, cortex	-	3	-
Basophilic tubules, diffuse	-	3	-
Inflammation, chronic	-	2	-
SMALL INTESTINE, DUODENUM	-	-	-

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 3: 30 ug/kg body weight
SEX: MALE

ANIMAL ID:	1259	1261	1265
Dilatation, mucosal gland	-	2	-
Congestion	-	2	-
SPLEEN	-	-	-
Mineralization, artery	-	2	-
PANCREAS	-	N	-
LYMPH NODE,MESENTERIC	-	-	-
Depletion, lymphoid	-	2	-
LIVER	-	-	-
Inflammation, chronic, periportal	-	1	-
GALLBLADDER	-	N	-
LARGE INTESTINE,RECTUM	-	-	-
Congestion	-	2	-
ADRENAL GLAND	-	N	-
PERIPHERAL NERVE,SCIATIC	-	N	-
SALIVARY GLAND	-	N	-
TONGUE	-	N	-
LYMPH NODE,MANDIBULAR	-	-	-
Sinus erythrocytosis	-	1	-
SKIN,ELBOW	-	N	-
SMALL INTESTINE,JEJUNUM	-	-	-
Dilatation, crypt glands	-	1	-
Congestion	-	2	-

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

DRAFT

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 3: 30 ug/kg body weight
SEX: MALE

ANIMAL ID:	1259	1261	1265
LARGE INTESTINE, COLON	-	-	-
Dilatation, crypt glands	-	2	-
Congestion	-	2	-
TONSIL	-	-	-
Mineralization, focal	-	2	-
Inflammation, subacute	-	1	-
Hemorrhage	-	2	-
SKIN, DORSAL THORAX	-	N	-
SMALL INTESTINE, ILEUM	-	-	-
Congestion	-	2	-
THYMUS	-	-	-
Atrophy	-	4	-
SKELETAL MUSCLE	-	-	-
Atrophy	-	3	-
SKIN	-	-	-
Ulceration	-	4	-
MAMMARY GLAND	-	N	-
THYROID GLAND	-	-	-
Hypertrophy/hyperplasia, parafollicular cell	-	3	-
PARATHYROID GLAND	-	-	-
Cyst	-	1	-
Hypertrophy	-	2	-
PITUITARY GLAND	-	N	-
URETER	-	N	-

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

DRAFT

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 3: 30 ug/kg body weight
SEX: MALE

ANIMAL ID:	1259	1261	1265
STOMACH	-	-	-
Accumulation, lymphocyte	-	1	-
Mineralization, mid-mucosal, pyloric	-	3	-
LARGE INTESTINE, CECUM	-	N	-
URINARY BLADDER	-	N	-
TESTES	-	-	-
Sexual immaturity	-	P	-
EPIDIDYMIS	-	-	-
Oligospermia	-	4	-
PROSTATE	-	-	-
Sexual immaturity	-	P	-
EYE	-	N	-
OPTIC NERVE	-	N	-
BONE, FEMUR	-	-	-
Hypoplasia, epiphyseal cartilage	-	2	-
BONE MARROW, FEMORAL	-	N	-
BONE, STERNUM	-	N	-
BONE MARROW, STERNUM	-	N	-

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

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PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 1: 0 ug/kg body weight
SEX: FEMALE

ANIMAL ID:	1235	1245	1249
BRAIN, FORE	N	N	N
SPINAL CORD, CERVICAL	N	N	N
BRAIN, MID	N	N	N
SPINAL CORD, THORACIC	N	N	N
BRAIN, HIND	N	N	N
HEART	N	-	-
Inflammation, chronic, artery, auricle	-	2	-
Hyperplasia, serosa, focal	-	-	2
TRACHEA	N	N	N
ESOPHAGUS	N	N	N
AORTA	N	N	N
LYMPH NODE, BRONCHIAL	-	-	-
Sinus erythrocytosis	3	1	1
LUNG	-	-	-
Inflammation, subacute, focal	-	2	-
Inflammation, chronic, perivascular	-	1	1
Hemorrhage, acute, focal	3	-	-
Edema	3	-	-
KIDNEY	-	-	-
Mineralization, medulla	1	1	2
SMALL INTESTINE, DUODENUM	N	N	N
SPLEEN	N	N	N

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

DRAFT

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 1: 0 ug/kg body weight
SEX: FEMALE

ANIMAL ID:	1235	1245	1249
PANCREAS	N	N	N
LYMPH NODE, MESENTERIC	-	-	-
Sinus erythrocytosis	1	1	1
LIVER	-	-	-
Inflammation, chronic, periportal	1	1	1
Inflammation, chronic, focal	1	1	1
GALLBLADDER	-	N	-
Accumulation, lymphocyte	1	-	1
LARGE INTESTINE, RECTUM	-	N	N
Dilatation, crypt glands	1	-	-
ADRENAL GLAND	N	N	N
PERIPHERAL NERVE, SCIATIC	N	N	N
SALIVARY GLAND	N	N	-
Inflammation, chronic	-	-	1
TONGUE	-	-	-
Inflammation, chronic, perivascular	2	1	1
LYMPH NODE, MANDIBULAR	N	-	N
Granulopoiesis	-	1	-
SKIN, ELBOW	N	-	N
Inflammation, subacute, dermis	-	2	-
SMALL INTESTINE, JEJUNUM	N	N	N
LARGE INTESTINE, COLON	N	N	N

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 1: 0 ug/kg body weight
SEX: FEMALE

ANIMAL ID:	1235	1245	1249
TONSIL	-	-	-
Mineralization, focal	1	-	1
Inflammation, subacute	1	1	1
Hemorrhage	2	-	-
SKIN,DORSAL THORAX	N	N	N
SMALL INTESTINE,ILEUM	N	N	N
THYMUS	N	N	N
SKELETAL MUSCLE	N	N	-
Inflammation, chronic, focal	-	-	1
SKIN	N	N	N
MAMMARY GLAND	U	U	N
THYROID GLAND	N	N	N
PARATHYROID GLAND	-	N	-
Cyst	1	-	1
PITUITARY GLAND	N	N	N
URETER	N	N	N
STOMACH	-	N	-
Mineralization, focal	2	-	-
Accumulation, lymphocyte	-	-	2
LARGE INTESTINE,CECUM	N	N	-
Congestion	-	-	1
URINARY BLADDER	-	N	-

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

DRAFT

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 1: 0 ug/kg body weight
SEX: FEMALE

ANIMAL ID:	1235	1245	1249
Accumulation, lymphocyte	2	-	-
Inflammation, subacute	2	-	-
Inflammation, chronic, perivascular	-	-	2
OVARY	N	N	N
FALLOPIAN TUBE	N	N	U
UTERUS	N	N	N
VAGINA	N	N	N
CERVIX	N	N	N
EYE	N	N	N
OPTIC NERVE	N	N	N
BONE, FEMUR	N	N	N
BONE MARROW, FEMORAL	N	N	N
BONE, STERNUM	N	N	N
BONE MARROW, STERNUM	N	N	N
Non-Protocol Tissues:			
LYMPH NODE, MEDIASTINAL	-	-	-
Sinus erythrocytosis	-	3	3

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

DRAFT

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 5: 5 ug/kg body weight
SEX: FEMALE

ANIMAL ID:	1236	1244
HEART	N	N
KIDNEY	-	-
Mineralization, medulla	1	2
Basophilic tubules	-	1
Dilatation, tubules	1	-
Mineralization, cortex	1	-
Basophilic tubules, diffuse	1	-
Inflammation, chronic	2	-
SPLEEN	N	N
ADRENAL GLAND	N	N
SALIVARY GLAND	N	N
SKIN, ELBOW	N	-
Inflammation, subacute, dermis	-	1
SKIN, DORSAL THORAX	N	-
Inflammation, chronic, hair follicle	-	1
THYMUS	-	-
Atrophy	1	2
Hemorrhage, serosal	-	3
SKELETAL MUSCLE	N	N
SKIN	N	-
MAMMARY GLAND	N	-
THYROID GLAND	N	-
Hypertrophy/hyperplasia, parafollicular cell	-	1

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 5: 5 ug/kg body weight
SEX: FEMALE

ANIMAL ID:	1236	1244
PARATHYROID GLAND	N	N
STOMACH	-	-
Accumulation, lymphocyte	2	2
Mineralization, mid-mucosal, pyloric	2	-
OVARY	N	N
UTERUS	N	N
BONE, FEMUR	N	N
BONE MARROW, FEMORAL	N	N
BONE, STERNUM	N	N
BONE MARROW, STERNUM	N	N

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

DRAFT

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 2: 10 ug/kg body weight
SEX: FEMALE

ANIMAL ID:	1242	1246	1250
BRAIN, FORE	N	-	N
Hemorrhage, acute, perivascular	-	1	-
SPINAL CORD, CERVICAL	N	-	-
Hemorrhage, acute, perivascular	-	1	1
BRAIN, MID	N	N	N
SPINAL CORD, THORACIC	N	N	N
BRAIN, HIND	N	N	N
HEART	N	N	N
TRACHEA	N	N	N
ESOPHAGUS	N	N	N
AORTA	N	N	N
LYMPH NODE, BRONCHIAL	N	N	N
LUNG	-	-	N
Inflammation, subacute, focal	1	-	-
Inflammation, chronic, perivascular	-	1	-
KIDNEY	-	-	-
Mineralization, medulla	1	1	1
Dilatation, tubules	3	3	4
Mineralization, cortex	2	2	2
Basophilic tubules, diffuse	3	3	3
Inflammation, chronic	-	1	-
SMALL INTESTINE, DUODENUM	N	-	-
Dilatation, mucosal gland	-	3	1

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

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PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 2: 10 ug/kg body weight
SEX: FEMALE

ANIMAL ID:	1242	1246	1250
SPLEEN	N	-	-
Mineralization, artery	-	2	1
PANCREAS	N	N	N
LYMPH NODE, MESENTERIC	-	N	N
Sinus erythrocytosis	1	-	-
LIVER	-	-	-
Inflammation, chronic, periportal	1	1	1
Inflammation, chronic, focal	1	1	1
GALLBLADDER	N	N	-
Accumulation, lymphocyte	-	-	1
LARGE INTESTINE, RECTUM	N	-	-
Dilatation, crypt glands	-	1	-
Congestion	-	-	1
ADRENAL GLAND	-	N	N
Mineralization, cortex, focal	2	-	-
PERIPHERAL NERVE, SCIATIC	N	N	N
SALIVARY GLAND	N	N	N
TONGUE	-	-	-
Inflammation, chronic, perivascular	1	1	1
LYMPH NODE, MANDIBULAR	N	-	N
Tattoo pigment	-	P	-
SKIN, ELBOW	N	N	N
SMALL INTESTINE, JEJUNUM	N	N	N

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 2: 10 ug/kg body weight
SEX: FEMALE

ANIMAL ID:	1242	1246	1250
LARGE INTESTINE, COLON	N	N	N
TONSIL	-	-	-
Mineralization, focal	1	1	1
Inflammation, subacute	2	1	1
Hemorrhage	-	-	1
SKIN, DORSAL THORAX	N	N	N
SMALL INTESTINE, ILEUM	N	N	N
THYMUS	-	-	-
Atrophy	3	2	3
SKELETAL MUSCLE	-	-	-
Atrophy	2	2	2
SKIN	N	N	N
MAMMARY GLAND	N	U	N
THYROID GLAND	-	-	N
Hypertrophy/hyperplasia, parafollicular cell	1	1	-
PARATHYROID GLAND	-	-	-
Cyst	-	2	-
Hypertrophy	1	1	1
PITUITARY GLAND	N	N	N
URETER	N	N	N
STOMACH	-	-	N
Accumulation, lymphocyte	2	-	-
Mineralization, mid-mucosal, pyloric	3	1	-

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

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PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 2: 10 ug/kg body weight
SEX: FEMALE

ANIMAL ID:	1242	1246	1250
LARGE INTESTINE, CECUM	-	N	N
Dilatation, crypt gland	1	-	-
URINARY BLADDER	N	N	N
OVARY	N	N	N
FALLOPIAN TUBE	N	N	N
UTERUS	-	-	-
Atrophy	2	2	3
VAGINA	N	N	N
CERVIX	N	N	N
EYE	N	N	N
OPTIC NERVE	N	N	N
BONE, FEMUR	-	-	-
Hypoplasia, epiphyseal cartilage	2	2	2
BONE MARROW, FEMORAL	-	-	-
Depletion	3	3	1
BONE, STERNUM	N	N	N
BONE MARROW, STERNUM	-	-	N
Depletion	1	1	-
Non-Protocol Tissues:			
LYMPH NODE, MEDIASTINAL	-	-	-
Sinus erythrocytosis	3	-	-

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

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PATHOLOGY ASSOCIATES
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1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 2: 10 ug/kg body weight
SEX: FEMALE

ANIMAL ID:	1242	1246	1250
Non-Protocol Tissues:			
MESENTERY	-	-	-
Cyst, blood	-	2	-

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

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PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 3: 30 ug/kg body weight
SEX: FEMALE

ANIMAL ID:	1238	1239	1243
BRAIN, FORE	-	N	-
SPINAL CORD, CERVICAL	-	N	-
BRAIN, MID	-	N	-
SPINAL CORD, THORACIC	-	N	-
BRAIN, HIND	-	N	-
HEART	-	N	-
TRACHEA	-	N	-
ESOPHAGUS	-	N	-
AORTA	-	N	-
LYMPH NODE, BRONCHIAL	-	-	-
Sinus erythrocytosis	-	2	-
Depletion, lymphoid	-	2	-
LUNG	-	-	-
Inflammation, subacute, focal	-	3	-
KIDNEY	-	-	-
Mineralization, medulla	-	2	-
Dilatation, tubules	-	3	-
Mineralization, cortex	-	3	-
Basophilic tubules, diffuse	-	3	-
Congestion	-	2	-
SMALL INTESTINE, DUODENUM	-	-	-
Congestion	-	2	-

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

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PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 3: 30 ug/kg body weight
SEX: FEMALE

ANIMAL ID:	1238	1239	1243
SPLEEN	-	N	-
PANCREAS	-	N	-
LYMPH NODE, MESENTERIC	-	-	-
Sinus erythrocytosis	-	3	-
Depletion, lymphoid	-	2	-
LIVER	-	-	-
Inflammation, chronic, periportal	-	2	-
GALLBLADDER	-	N	-
LARGE INTESTINE, RECTUM	-	-	-
Dilatation, crypt glands	-	2	-
Congestion	-	2	-
ADRENAL GLAND	-	-	-
Vacuolation, cortex	-	2	-
PERIPHERAL NERVE, SCIATIC	-	N	-
SALIVARY GLAND	-	N	-
TONGUE	-	-	-
Inflammation, subacute, focal	-	2	-
Erosion, focal	-	2	-
LYMPH NODE, MANDIBULAR	-	-	-
Tattoo pigment	-	P	-
SKIN, ELBOW	-	N	-
SMALL INTESTINE, JEJUNUM	-	-	-
Congestion	-	1	-

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

DRAFT

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 3: 30 ug/kg body weight
SEX: FEMALE

ANIMAL ID:	1238	1239	1243
LARGE INTESTINE, COLON	-	-	-
Dilatation, crypt glands	-	2	-
Congestion	-	2	-
TONSIL	-	-	-
Mineralization, focal	-	1	-
Inflammation, subacute	-	1	-
Hemorrhage	-	2	-
SKIN, DORSAL THORAX	-	N	-
SMALL INTESTINE, ILEUM	-	-	-
Congestion	-	2	-
THYMUS	-	-	-
Atrophy	-	4	-
SKELETAL MUSCLE	-	-	-
Atrophy	-	3	-
Degeneration	-	3	-
Inflammation, subacute	-	2	-
SKIN	-	N	-
MAMMARY GLAND	-	N	-
THYROID GLAND	-	-	-
Hypertrophy/hyperplasia, parafollicular cell	-	3	-
PARATHYROID GLAND	-	N	-
PITUITARY GLAND	-	-	-
Cyst	-	1	-
URETER	-	N	-

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

DRAFT

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 3: 30 ug/kg body weight
SEX: FEMALE

ANIMAL ID:	1238	1239	1243
STOMACH	-	-	-
Accumulation, lymphocyte	-	1	-
Mineralization, mid-mucosal, pyloric	-	4	-
Congestion	-	2	-
LARGE INTESTINE,CECUM	-	-	-
Dilatation, crypt gland	-	1	-
Congestion	-	2	-
URINARY BLADDER	-	N	-
OVARY	-	N	-
FALLOPIAN TUBE	-	N	-
UTERUS	-	-	-
Atrophy	-	3	-
VAGINA	-	N	-
CERVIX	-	N	-
EYE	-	N	-
OPTIC NERVE	-	N	-
BONE,FEMUR	-	-	-
Hypoplasia, epiphyseal cartilage	-	2	-
BONE MARROW,FEMORAL	-	-	-
Depletion	-	3	-
BONE,STERNUM	-	N	-
BONE MARROW,STERNUM	-	-	-

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

DRAFT

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

TABULATED ANIMAL DATA

STUDY ID : 1209 SN2
FATE: ALL
DAYS ON TEST: ALL

STUDY NUMBER: 1209SN2
GROUP: 3: 30 ug/kg body weight
SEX: FEMALE

ANIMAL ID:	1238	1239	1243
Depletion	-	3	-

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

19-JAN-2001

SECTION V

CORRELATION OF GROSS AND MICROSCOPIC (MICRO) FINDINGS

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

CORRELATION OF GROSS & MICRO

STUDY ID: 1209 SN2
SEX: MALE

STUDY NUMBER: 1209SN2
GROUP: 1: 0 ug/kg body weight

Animal ID: 1252
Animal Fate: Terminal sacrifice
Days on Test: 37

Reference to Necropsy Record:	Related Histopathology:
LYMPH NODE,BRONCHIAL - PIGMENTATION, MOTTLED	LYMPH NODE,BRONCHIAL - Sinus erythrocytosis
PROSTATE - SMALL	PROSTATE - Sexual immaturity

Animal ID: 1256
Animal Fate: Terminal sacrifice
Days on Test: 37

Reference to Necropsy Record:	Related Histopathology:
TONSIL - BILATERAL, PIGMENTATION, RED	TONSIL - Hemorrhage
PROSTATE - SMALL	PROSTATE - Sexual immaturity
LYMPH NODE,MEDIASTINAL - PIGMENTATION, DARK	LYMPH NODE,MEDIASTINAL - Sinus erythrocytosis

Animal ID: 1263
Animal Fate: Terminal sacrifice
Days on Test: 37

Reference to Necropsy Record:	Related Histopathology:
LYMPH NODE,MEDIASTINAL - PIGMENTATION, RED	LYMPH NODE,MEDIASTINAL - Sinus erythrocytosis
PROSTATE - SMALL	PROSTATE - Sexual immaturity
LYMPH NODE,MESENTERIC - PIGMENTATION, RED	LYMPH NODE,MESENTERIC - Sinus erythrocytosis

LABCAT HP4.33

19-JAN-2001

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

CORRELATION OF GROSS & MICRO

STUDY ID: 1209 SN2
SEX: MALE

STUDY NUMBER: 1209SN2
GROUP: 5: 5 ug/kg body weight

Animal ID: 1258
Animal Fate: Terminal sacrifice
Days on Test: 29

Reference to Necropsy Record:	Related Histopathology:
TESTES - BILATERAL, SMALL	TESTES - Sexual immaturity
TONSIL - BILATERAL, PIGMENTATION, RED	TONSIL - Hemorrhage
PROSTATE - SMALL	PROSTATE - Sexual immaturity
BONE - LEFT, LESION, (OCCIPITAL MISSING)	BONE - No specimen taken

Animal ID: 1266
Animal Fate: Terminal sacrifice
Days on Test: 29

Reference to Necropsy Record:	Related Histopathology:
TONSIL - BILATERAL, PIGMENTATION, RED	TONSIL - Hemorrhage
PROSTATE - SMALL	PROSTATE - Sexual immaturity
SMALL INTESTINE,ILEUM - PIGMENTATION, RED	SMALL INTESTINE,ILEUM - No corresponding lesion
LARGE INTESTINE,CECUM - PIGMENTATION, RED	LARGE INTESTINE,CECUM - No corresponding lesion

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PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

CORRELATION OF GROSS & MICRO

STUDY ID: 1209 SN2
SEX: MALE

STUDY NUMBER: 1209SN2
GROUP: 2: 10 ug/kg body weight

Animal ID: 1257
Animal Fate: Terminal sacrifice

Days on Test: 29

Reference to Necropsy Record:	Related Histopathology:
PROSTATE - SMALL	PROSTATE - Sexual immaturity
TESTES - BILATERAL, SMALL	TESTES - Sexual immaturity
EPIDIDYMIS - BILATERAL, SMALL	EPIDIDYMIS - Oligospermia
TONSIL - BILATERAL, PIGMENTATION, RED	TONSIL - Hemorrhage
THYMUS - SMALL	THYMUS - Atrophy
KIDNEY - MEDULLA, BILATERAL, PIGMENTATION, RED	KIDNEY - Congestion

Animal ID: 1260
Animal Fate: Terminal sacrifice

Days on Test: 29

Reference to Necropsy Record:	Related Histopathology:
TESTES - BILATERAL, SMALL	TESTES - Sexual immaturity
EPIDIDYMIS - BILATERAL, SMALL	EPIDIDYMIS - Oligospermia
TONSIL - BILATERAL, PIGMENTATION, RED	TONSIL - Hemorrhage
LYMPH NODE,MANDIBULAR - BILATERAL,PIGMENTATION, DARK	LYMPH NODE,MANDIBULAR - Tattoo pigment
THYMUS - SMALL	THYMUS - Atrophy
PROSTATE - SMALL	PROSTATE - Sexual immaturity
LYMPH NODE,DEEP CERVICAL - PIGMENTATION, DARK	LYMPH NODE,DEEP CERVICAL - Sinus erythrocytosis
SMALL INTESTINE,DUODENUM - PIGMENTAION, DARK	SMALL INTESTINE,DUODENUM - No corresponding lesion
KIDNEY - MEDULLA, BILATERAL, PIGMENTATION, RED	KIDNEY - Congestion

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FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

CORRELATION OF GROSS & MICRO

STUDY ID: 1209 SN2
SEX: MALE

STUDY NUMBER: 1209SN2
GROUP: 2: 10 ug/kg body weight

Animal ID: 1262
Animal Fate: Terminal sacrifice

Days on Test: 29

Reference to Necropsy Record:	Related Histopathology:
SKIN - RIGHT, FACE, THICK, (MUCOSA,ULCERATED)	SKIN - Abscess; Bacteria; Ulceration
LYMPH NODE,MANDIBULAR - RIGHT, ENLARGED	LYMPH NODE,MANDIBULAR - Sinus erythrocytosis
TONSIL - BILATERAL, PIGMENTATION, RED	TONSIL - Hemorrhage
TESTES - BILATERAL, SMALL	TESTES - Sexual immaturity
EPIDIDYMIS - BILATERAL, SMALL	EPIDIDYMIS - Oligospermia
THYMUS - SMALL	THYMUS - Atrophy
LYMPH NODE,DEEP CERVICAL - RIGHT, ENLARGED	LYMPH NODE,DEEP CERVICAL - Hyperplasia, lymphoid
PROSTATE - SMALL	PROSTATE - Sexual immaturity
SMALL INTESTINE,JEJUNUM - PIGMENTATION, RED	SMALL INTESTINE,JEJUNUM - No corresponding lesion
SMALL INTESTINE,ILEUM - PIGMENTATION, RED	SMALL INTESTINE,ILEUM - No corresponding lesion
LARGE INTESTINE,COLON - PIGMENTATION, RED	LARGE INTESTINE,COLON - Congestion
LARGE INTESTINE,CECUM - PIGMENTATION, RED	LARGE INTESTINE,CECUM - Congestion
LARGE INTESTINE,RECTUM - PIGMENTATION, RED	LARGE INTESTINE,RECTUM - Congestion
KIDNEY - PELVIS, LEFT, DILATATION	KIDNEY - Dilatation, pelvis
KIDNEY - BILATERAL, MEDULLA, PIGMENTATION, RED	KIDNEY - Congestion

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FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

CORRELATION OF GROSS & MICRO

STUDY ID: 1209 SN2
SEX: MALE

STUDY NUMBER: 1209SN2
GROUP: 3: 30 ug/kg body weight

Animal ID: 1259
Animal Fate: Natural death
Days on Test: 24

Reference to Necropsy Record:	Related Histopathology:
SKIN - FACE, PIGMENTATION, BLACK, (BILATERAL, CHEEK)	SKIN - Not required by protocol
TONSIL - BILATERAL, PIGMENTATION, DARK RED	TONSIL - Not required by protocol
TONGUE - PIGMENTATION, RED, MOTTLED	TONGUE - Not required by protocol
LUNG - CARDIAC LOBE, PIGMENTATION, MOTTLED	LUNG - Not required by protocol
LUNG - LEFT, FOCUS, 5X5 MM, MULTIPLE, WHITE, (FOCI ON LUNG PERIPHERY)	LUNG - Not required by protocol
THYMUS - PARENCHYMA, SMALL	THYMUS - Not required by protocol
STOMACH - PYLORIC, PIGMENTATION, RED	STOMACH - Not required by protocol
SMALL INTESTINE,DUODENUM - PIGMENTATION, RED	SMALL INTESTINE,DUODENUM - Not required by protocol
SMALL INTESTINE,JEJUNUM - PIGMENTATION, RED	SMALL INTESTINE,JEJUNUM - Not required by protocol
SMALL INTESTINE,ILEUM - FOCUS, 10X5 MM, RED, (ULCERATION)	SMALL INTESTINE,ILEUM - Not required by protocol
LARGE INTESTINE,COLON - PIGMENTATION, RED	LARGE INTESTINE,COLON - Not required by protocol
LARGE INTESTINE,RECTUM - PIGMENTATION, DARK RED	LARGE INTESTINE,RECTUM - Not required by protocol

Animal ID: 1261
Animal Fate: Moribund sacrifice
Days on Test: 24

Reference to Necropsy Record:	Related Histopathology:
LYMPH NODE,MANDIBULAR - ENLARGED	LYMPH NODE,MANDIBULAR - No corresponding lesion
SKIN - FACE, PIGMENTATION, BLACK, (BILATERAL, CHEEK)	SKIN - Ulceration
TONSIL - BILATERAL, PIGMENTATION, RED	TONSIL - Hemorrhage
SMALL INTESTINE,DUODENUM - PIGMENTATION, RED	SMALL INTESTINE,DUODENUM - Congestion

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FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

CORRELATION OF GROSS & MICRO

STUDY ID: 1209 SN2
SEX: MALE

STUDY NUMBER: 1209SN2
GROUP: 3: 30 ug/kg body weight

Animal ID: 1261
Animal Fate: Moribund sacrifice

Days on Test: 24

Reference to Necropsy Record:	Related Histopathology:
SMALL INTESTINE,JEJUNUM - PIGMENTATION, RED	SMALL INTESTINE,JEJUNUM - Congestion
SMALL INTESTINE,ILEUM - PIGMENTATION, RED	SMALL INTESTINE,ILEUM - Congestion
LARGE INTESTINE,RECTUM - PIGMENTATION, RED	LARGE INTESTINE,RECTUM - Congestion
THYMUS - PARENCHYMA, SMALL	THYMUS - Atrophy
TESTES - BILATERAL, SMALL	TESTES - Sexual immaturity
EPIDIDYMIS - BILATERAL, SMALL	EPIDIDYMIS - Oligospermia

Animal ID: 1265
Animal Fate: Terminal sacrifice

Days on Test: 29

Reference to Necropsy Record:	Related Histopathology:
TESTES - BILATERAL, SMALL	TESTES - Not required by portocol
EPIDIDYMIS - BILATERAL, SMALL	EPIDIDYMIS - Not required by protocol
THYMUS - SMALL	THYMUS - Not required by protocol
PROSTATE - SMALL	PROSTATE - Not required by protocol
LUNG - RIGHT DIAPHRAGMATIC LOBE, FOCUS 10X15 MM, BROWN	LUNG - Not required by protocol
LUNG - LEFT, FOCUS, 10X15 MM, BROWN	LUNG - Not required by protocol
SMALL INTESTINE,DUODENUM - PIGMENTATION, RED	SMALL INTESTINE,DUODENUM - Not required by protocol
SMALL INTESTINE,JEJUNUM - PIGMENTATION, RED	SMALL INTESTINE,JEJUNUM - Not required by protocol
LARGE INTESTINE,COLON - PIGMENTATION,RED	LARGE INTESTINE,COLON - Not required by protocol

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FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

CORRELATION OF GROSS & MICRO

STUDY ID: 1209 SN2
SEX: MALE

STUDY NUMBER: 1209SN2
GROUP: 3: 30 ug/kg body weight

Animal ID: 1265
Animal Fate: Terminal sacrifice

Days on Test: 29

Reference to Necropsy Record:	Related Histopathology:
KIDNEY - RENAL PELVIS, BILATERAL, PIGMENTATION, RED	KIDNEY - Not required by protocol

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FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

CORRELATION OF GROSS & MICRO

STUDY ID: 1209 SN2
SEX: MALE

STUDY NUMBER: 1209SN2
GROUP: 4: 45 ug/kg body weight

Animal ID: 1251
Animal Fate: Natural death

Days on Test: 27

Reference to Necropsy Record:	Related Histopathology:
TESTES - BILATERAL, SMALL	TESTES - Not required by protocol
EPIDIDYMIS - BILATERAL, SMALL	EPIDIDYMIS - Not required by protocol
TONSIL - BILATERAL, PIGMENTATION, DARK RED	TONSIL - Not required by protocol
THYMUS - PIGMENTATION, DARK	THYMUS - Not required by protocol
THYMUS - SMALL	THYMUS - Not required by protocol
SPLEEN - PIGMENTATION, PALE	SPLEEN - Not required by protocol
SMALL INTESTINE,DUODENUM - PIGMENTATION, RED	SMALL INTESTINE,DUODENUM - Not required by protocol
STOMACH - CARDIAC, PIGMENTATION, RED	STOMACH - Not required by protocol
STOMACH - FUNDIC, PIGMENTATION, RED	STOMACH - Not required by protocol
STOMACH - PYLORIC, PIGMENTATION, RED	STOMACH - Not required by protocol
SMALL INTESTINE,JEJUNUM - PIGMENTATION, RED	SMALL INTESTINE,JEJUNUM - Not required by protocol
LARGE INTESTINE,CECUM - PIGMENTATION, RED	LARGE INTESTINE,CECUM - Not required by protocol
LARGE INTESTINE,COLON - PIGMENTATION, RED	LARGE INTESTINE,COLON - Not required by protocol
LARGE INTESTINE,RECTUM - PIGMENTATION, RED	LARGE INTESTINE,RECTUM - Not required by protocol
PROSTATE - SMALL	PROSTATE - Not required by protocol
LUNG - CARDIAC LOBE, PIGMENTATION, MOTTLED	LUNG - Not required by protocol
LUNG - LEFT, PIGMENTATION, MOTTLED	LUNG - Not required by protocol

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FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

CORRELATION OF GROSS & MICRO

STUDY ID: 1209 SN2
SEX: MALE

STUDY NUMBER: 1209SN2
GROUP: 4: 45 ug/kg body weight

Animal ID: 1253
Animal Fate: Moribund sacrifice

Days on Test: 23

Reference to Necropsy Record:	Related Histopathology:
THYMUS - PARENCHYMA, SMALL	THYMUS - Not required by protocol
LUNG - CARDIAC LOBE, MASS, 15X15 MM, DARK RED	LUNG - Not required by protocol
LUNG - CARDAIC LOBE, PIGMENTATION, RED	LUNG - Not required by protocol
LUNG - LEFT, MASS 18X18 MM, DARK RED	LUNG - Not required by protocol
LUNG - LEFT, PIGMENTATION, RED	LUNG - Not required by protocol
SMALL INTESTINE,DUODENUM - PIGMENTATION,RED	SMALL INTESTINE,DUODENUM - Not required by protocol
SMALL INTESTINE,JEJUNUM - PIGMENTATION, RED	SMALL INTESTINE,JEJUNUM - Not required by protocol
LARGE INTESTINE,CECUM - PIGMENTATION, RED	LARGE INTESTINE,CECUM - Not required by protocol
LARGE INTESTINE,COLON - PIGMENTATION, RED	LARGE INTESTINE,COLON - Not required by protocol
LARGE INTESTINE,RECTUM - PIGMENTATION, RED	LARGE INTESTINE,RECTUM - Not required by protocol

Animal ID: 1254
Animal Fate: Terminal sacrifice

Days on Test: 30

Reference to Necropsy Record:	Related Histopathology:
LYMPH NODE,MEDIASTINAL - PIGMENTATION, DARK	LYMPH NODE,MEDIASTINAL - Not required by protocol
THYMUS - SMALL	THYMUS - Not required by protocol
TESTES - SMALL	TESTES - Not required by protocol
EPIDIDYMIS - SMALL	EPIDIDYMIS - Not required by protocol
PROSTATE - SMALL	PROSTATE - Not required by protocol
SPLEEN - PIGMENTATION, PALE	SPLEEN - Not required by protocol
SPLEEN - FOCUS, 8X8 MM, MOTTLED	SPLEEN - Not required by protocol

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FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

CORRELATION OF GROSS & MICRO

STUDY ID: 1209 SN2 | STUDY NUMBER: 1209SN2
SEX: MALE | GROUP: 4: 45 ug/kg body weight

Animal ID: 1254
Animal Fate: Terminal sacrifice | Days on Test: 30

Reference to Necropsy Record:	Related Histopathology:
SMALL INTESTINE,JEJUNUM - PIGMENTATION, RED	SMALL INTESTINE,JEJUNUM - Not required by protocol
THYROID GLAND - BILATERAL, PIGMENTATION, PALE	THYROID GLAND - Not required by protocol
KIDNEY - BILATERAL, PIGMENTATION, PALE	KIDNEY - Not required by protocol

Animal ID: 1255
Animal Fate: Natural death | Days on Test: 23

Reference to Necropsy Record:	Related Histopathology:
THYMUS - PARENCHYMA, SMALL	THYMUS - Not required by protocol
LUNG - PARENCHYMA, PIGMENTATION, RED	LUNG - Not required by protocol
STOMACH - CARDIAC, PIGMENTATION, RED	STOMACH - Not required by protocol
STOMACH - FUNDIC, PIGMENTATION, RED	STOMACH - Not required by protocol
STOMACH - PYLORIC, PIGMENTATION, RED	STOMACH - Not required by protocol
SMALL INTESTINE,DUODENUM - PIGMENTATION, RED	SMALL INTESTINE,DUODENUM - Not required by protocol
SMALL INTESTINE,JEJUNUM - PIGMENTATION, RED	SMALL INTESTINE,JEJUNUM - Not required by protocol
LARGE INTESTINE,RECTUM - PIGMENTATION, RED	LARGE INTESTINE,RECTUM - Not required by protocol

Animal ID: 1264
Animal Fate: Terminal sacrifice | Days on Test: 30

Reference to Necropsy Record:	Related Histopathology:
SKIN - FACE, LEFT, THICK, DARK, (MUCOSAL SURFACE ULCERATED)	SKIN - Not required by protocol
LYMPH NODE,MANDIBULAR - LEFT, ENLARGED	LYMPH NODE,MANDIBULAR - Not required by protocol

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

CORRELATION OF GROSS & MICRO

STUDY ID: 1209 SN2
SEX: MALE

STUDY NUMBER: 1209SN2
GROUP: 4: 45 ug/kg body weight

Animal ID: 1264
Animal Fate: Terminal sacrifice

Days on Test: 30

Reference to Necropsy Record:	Related Histopathology:
TESTES - SMALL	TESTES - Not required by protocol
EPIDIDYMIS - SMALL	EPIDIDYMIS - Not required by protocol
LYMPH NODE,MEDIASTINAL - PIGMENTATION, DARK	LYMPH NODE,MEDIASTINAL - Not required by protocol
PROSTATE - SMALL	PROSTATE - Not required by protocol
THYMUS - SMALL	THYMUS - Not required by protocol
THYROID GLAND - BILATERAL, PIGMENTATION, PALE	THYROID GLAND - Not required by protocol
SMALL INTESTINE,DUODENUM - PIGMENTATION, RED	SMALL INTESTINE,DUODENUM - Not required by protocol

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FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

CORRELATION OF GROSS & MICRO

STUDY ID: 1209 SN2
SEX: FEMALE

STUDY NUMBER: 1209SN2
GROUP: 1: 0 ug/kg body weight

Animal ID: 1235
Animal Fate: Terminal sacrifice

Days on Test: 36

Reference to Necropsy Record:
LYMPH NODE,BRONCHIAL - PIGMENTATION, DARK

Related Histopathology:
LYMPH NODE,BRONCHIAL - Sinus erythrocytosis

Animal ID: 1245
Animal Fate: Terminal sacrifice

Days on Test: 36

Reference to Necropsy Record:
LYMPH NODE,MEDIASTINAL - PIGMENTATION, DARK

Related Histopathology:
LYMPH NODE,MEDIASTINAL - Sinus erythrocytosis

Animal ID: 1249
Animal Fate: Terminal sacrifice

Days on Test: 36

Reference to Necropsy Record:
LYMPH NODE,MEDIASTINAL - PIGMENTATION, DARK

Related Histopathology:
LYMPH NODE,MEDIASTINAL - Sinus erythrocytosis

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FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

CORRELATION OF GROSS & MICRO

STUDY ID: 1209 SN2
SEX: FEMALE

STUDY NUMBER: 1209SN2
GROUP: 5: 5 ug/kg body weight

Animal ID: 1244
Animal Fate: Terminal sacrifice

Days on Test: 29

Reference to Necropsy Record:
THYMUS - BILATERAL, PIGMENTATION, DARK

Related Histopathology:
THYMUS - Hemorrhage, serosal

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FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

CORRELATION OF GROSS & MICRO

STUDY ID: 1209 SN2
SEX: FEMALE

STUDY NUMBER: 1209SN2
GROUP: 2: 10 ug/kg body weight

Animal ID: 1242
Animal Fate: Terminal sacrifice

Days on Test: 29

Reference to Necropsy Record:	Related Histopathology:
LYMPH NODE,MEDIASTINAL - PIGMENTATION, RED	LYMPH NODE,MEDIASTINAL - Sinus erythrocytosis
UTERUS - BILATERAL, SMALL	UTERUS - Atrophy
THYMUS - SMALL	THYMUS - Atrophy
KIDNEY - BILATERAL, PIGMENTATION, PALE	KIDNEY - Basophilic tubules, diffuse; Dilatation, tubules; Mineralization, cortex
SMALL INTESTINE,DUODENUM - PIGMENTATION, RED	SMALL INTESTINE,DUODENUM - No corresponding lesion

Animal ID: 1246
Animal Fate: Terminal sacrifice

Days on Test: 29

Reference to Necropsy Record:	Related Histopathology:
UTERUS - BILATERAL, SMALL	UTERUS - Atrophy
THYMUS - SMALL	THYMUS - Atrophy
MESENTERY - NODULE, 6X6X6 MM, BLACK	MESENTERY - Cyst, blood
SMALL INTESTINE,DUODENUM - PIGMENTATION, RED	SMALL INTESTINE,DUODENUM - No corresponding lesion
SMALL INTESTINE,JEJUNUM - PIGMENTATION, RED	SMALL INTESTINE,JEJUNUM - No corresponding lesion

Animal ID: 1250
Animal Fate: Terminal sacrifice

Days on Test: 29

Reference to Necropsy Record:	Related Histopathology:
UTERUS - BILATERAL, SMALL	UTERUS - Atrophy
SMALL INTESTINE,DUODENUM - PIGMENTATION, RED	SMALL INTESTINE,DUODENUM - No corresponding lesion
SMALL INTESTINE,ILEUM - PIGMENTATION, RED	SMALL INTESTINE,ILEUM - No corresponding lesion

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19-JAN-2001

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FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

CORRELATION OF GROSS & MICRO

STUDY ID: 1209 SN2
SEX: FEMALE

STUDY NUMBER: 1209SN2
GROUP: 2: 10 ug/kg body weight

Animal ID: 1250
Animal Fate: Terminal sacrifice

Days on Test: 29

Reference to Necropsy Record:	Related Histopathology:
LARGE INTESTINE, RECTUM - PIGMENTATION, RED	LARGE INTESTINE, RECTUM - Congestion
KIDNEY - BILATERAL, PIGMENTATION, PALE	KIDNEY - Dilatation, tubules; Mineralization, cortex; Basophilic tubules, diffuse
THYMUS - SMALL	THYMUS - Atrophy

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

CORRELATION OF GROSS & MICRO

STUDY ID: 1209 SN2 — STUDY NUMBER: 1209SN2
SEX: FEMALE — GROUP: 3: 30 ug/kg body weight

Animal ID: 1238 — Days on Test: 29
Animal Fate: Terminal sacrifice

Reference to Necropsy Record:	Related Histopathology:
OVARY - BILATERAL, SMALL	OVARY - Not required by protocol
UTERUS - BILATERAL, SMALL	UTERUS - Not required by protocol
THYMUS - SMALL	THYMUS - Not required by protocol
SMALL INTESTINE,JEJUNUM - PIGMENTATION, RED	SMALL INTESTINE,JEJUNUM - Not required by protocol
LARGE INTESTINE,COLON - PIGMENTATION, RED	LARGE INTESTINE,COLON - Not required by protocol

Animal ID: 1239 — Days on Test: 28
Animal Fate: Moribund sacrifice

Reference to Necropsy Record:	Related Histopathology:
OVARY - BILATERAL, SMALL	OVARY - No corresponding lesion
LYMPH NODE,MANDIBULAR - PIGMENTATION, DARK	LYMPH NODE,MANDIBULAR - Tattoo pigment
TONSIL - BILATERAL, PIGMENTATION, RED	TONSIL - Hemorrhage
THYMUS - SMALL	THYMUS - Atrophy
UTERUS - BILATERAL, SMALL	UTERUS - Atrophy
LYMPH NODE,BRONCHIAL - PIGMENTATION, DARK	LYMPH NODE,BRONCHIAL - Sinus erythrocytosis
LUNG - LEFT, CARDIAC LOBE, PIGMENTATION, MOTTLED	LUNG - Inflammation, subacute, focal
LUNG - RIGHT, CARDIAC LOBE, PIPGMENTATION, MOTTLED	LUNG - No corresponding lesion
STOMACH - PIGMENTATION, RED	STOMACH - Congestion
SMALL INTESTINE,DUODENUM - PIGMENTATION, RED	SMALL INTESTINE,DUODENUM - Congestion
SMALL INTESTINE,JEJUNUM - PIGMENTATION, RED	SMALL INTESTINE,JEJUNUM - Congestion
SMALL INTESTINE,ILEUM - PIGMENTATION, RED	SMALL INTESTINE,ILEUM - Congestion

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FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

CORRELATION OF GROSS & MICRO

STUDY ID: 1209 SN2
SEX: FEMALE

STUDY NUMBER: 1209SN2
GROUP: 3: 30 ug/kg body weight

Animal ID: 1239
Animal Fate: Moribund sacrifice

Days on Test: 28

Reference to Necropsy Record:	Related Histopathology:
LARGE INTESTINE,CECUM - PIGMENTATION, RED	LARGE INTESTINE,CECUM - Congestion
LARGE INTESTINE,COLON - PIGMENTATION, RED	LARGE INTESTINE,COLON - Congestion
LARGE INTESTINE,RECTUM - PIGMENTATION, RED	LARGE INTESTINE,RECTUM - Congestion
KIDNEY - MEDULLA, BILATERAL, PIGMENTATION, RED	KIDNEY - Congestion
LYMPH NODE,MESENTERIC - PIGMENTATION, DARK	LYMPH NODE,MESENTERIC - Sinus erythrocytosis

Animal ID: 1243
Animal Fate: Terminal sacrifice

Days on Test: 29

Reference to Necropsy Record:	Related Histopathology:
SMALL INTESTINE,DUODENUM - PIGMENTATION, RED	SMALL INTESTINE,DUODENUM - Not required by protocol
SMALL INTESTINE,JEJUNUM - PIGMENTATION, RED	SMALL INTESTINE,JEJUNUM - Not required by protocol
THYMUS - SMALL	THYMUS - Not required by protocol
UTERUS - BILATERAL, SMALL	UTERUS - Not required by protocol
LARGE INTESTINE,RECTUM - PIGMENTATION, RED	LARGE INTESTINE,RECTUM - Not required by protocol

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PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

CORRELATION OF GROSS & MICRO

STUDY ID: 1209 SN2
SEX: FEMALE

STUDY NUMBER: 1209SN2
GROUP: 4: 45 ug/kg body weight

Animal ID: 1237
Animal Fate: Terminal sacrifice

Days on Test: 30

Reference to Necropsy Record:	Related Histopathology:
THYMUS - SMALL	THYMUS - Not required by protocol
TONSIL - BILATERAL, PIGMENTATION, RED	TONSIL - Not required by protocol
UTERUS - BILATERAL, SMALL	UTERUS - Not required by protocol
THYROID GLAND - BILATERAL, PIGMENTATION, PALE	THYROID GLAND - Not required by protocol
SMALL INTESTINE,DUODENUM - PIGMENTATION, RED	SMALL INTESTINE,DUODENUM - Not required by protocol
LARGE INTESTINE,RECTUM - PIGMENTATION, RED	LARGE INTESTINE,RECTUM - Not required by protocol

Animal ID: 1240
Animal Fate: Terminal sacrifice

Days on Test: 30

Reference to Necropsy Record:	Related Histopathology:
LYMPH NODE,MEDIASTINAL - PIGMENTATION, DARK	LYMPH NODE,MEDIASTINAL - Not required by protocol
THYMUS - SMALL	THYMUS - Not required by protocol
UTERUS - BILATERAL, SMALL	UTERUS - Not required by protocol
SMALL INTESTINE,DUODENUM - PIGMENTATION,RED	SMALL INTESTINE,DUODENUM - Not required by protocol
SMALL INTESTINE,JEJUNUM - PIGMENTATION, RED	SMALL INTESTINE,JEJUNUM - Not required by protocol
THYROID GLAND - BILATERAL, PIGMENTATION, PALE	THYROID GLAND - Not required by protocol
LARGE INTESTINE,RECTUM - PIGMENTATION, RED	LARGE INTESTINE,RECTUM - Not required by protocol
KIDNEY - BILATERAL, PIGMENTATION, MOTTLED	KIDNEY - Not required by protocol

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PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

CORRELATION OF GROSS & MICRO

STUDY ID: 1209 SN2
SEX: FEMALE

STUDY NUMBER: 1209SN2
GROUP: 4: 45 ug/kg body weight

Animal ID: 1241
Animal Fate: Terminal sacrifice

Days on Test: 30

Reference to Necropsy Record:	Related Histopathology:
EYE - RIGHT, PIGMENTATION, OPAQUE	EYE - Not required by protocol
UTERUS - BILATERAL, SMALL	UTERUS - Not required by protocol
THYMUS - SMALL	THYMUS - Not required by protocol
LYMPH NODE,MEDIASTINAL - PIGMENTATION, DARK	LYMPH NODE,MEDIASTINAL - Not required by protocol
SMALL INTESTINE,DUODENUM - PIGMENTATION, RED	SMALL INTESTINE,DUODENUM - Not required by protocol
THYROID GLAND - BILATERAL, PIGMENTATION, PALE	THYROID GLAND - Not required by protocol
LARGE INTESTINE,RECTUM - PIGMENTATION, RED	LARGE INTESTINE,RECTUM - Not required by protocol

Animal ID: 1247
Animal Fate: Natural death

Days on Test: 7

Reference to Necropsy Record:	Related Histopathology:
THYMUS - PIGMENTATION,RED	THYMUS - Not required by protocol
SPLEEN - PIGMENTATION, PALE	SPLEEN - Not required by protocol
LYMPH NODE,MESENTERIC - PIGMENTATION,RED	LYMPH NODE,MESENTERIC - Not required by protocol
LUNG - APICAL LOBE, FOCUS, 10X10 MM, DARK, (LUNG FAILED TO COLLAPSE)	LUNG - Not required by protocol
LUNG - CARDIAC LOBE, FOCUS, 8X8 MM, DARK, (LUNG FAILED TO COLLAPSE)	LUNG - Not required by protocol
LUNG - DIAPHRAGMATIC LOBE, FOCUS, 14X15 MM, DARK	LUNG - Not required by protocol
STOMACH - CARDIAC, PIGMENTATION, MOTTLED	STOMACH - Not required by protocol
LYMPH NODE,BRONCHIAL - PIGMENTATION, MOTTLED	LYMPH NODE,BRONCHIAL - Not required by protocol
STOMACH - FUNDIC, PIGMENTATION, MOTTLED	STOMACH - Not required by protocol

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19-JAN-2001

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

CORRELATION OF GROSS & MICRO

STUDY ID: 1209 SN2
SEX: FEMALE

STUDY NUMBER: 1209SN2
GROUP: 4: 45 ug/kg body weight

Animal ID: 1247
Animal Fate: Natural death

Days on Test: 7

Reference to Necropsy Record:	Related Histopathology:
STOMACH - PYLORIC, PIGMENTATION, MOTTLED	STOMACH - Not required by protocol
SMALL INTESTINE,DUODENUM - PIGMENTATION, MOTTLED	SMALL INTESTINE,DUODENUM - Not required by protocol
LARGE INTESTINE,CECUM - PIGMENTATION, MOTTLED	LARGE INTESTINE,CECUM - Not required by protocol
LARGE INTESTINE,COLON - PIGMENTATION, MOTTLED	LARGE INTESTINE,COLON - Not required by protocol
LARGE INTESTINE,RECTUM - PIGMENTATION, MOTTLED	LARGE INTESTINE,RECTUM - Not required by protocol

Animal ID: 1248
Animal Fate: Natural death

Days on Test: 6

Reference to Necropsy Record:	Related Histopathology:
LUNG - LEFT DIAPHRAGMATIC LOBE, PIGMENTATION, MOTTLED, (LUNG FAILED TO COLLAPSE)	LUNG - Not required by protocol
LUNG - LEFT CARDIAC LOBE, PIGMENTATION, MOTTLED	LUNG - Nor required by protocol
LUNG - APICAL LOBE, PIGMENTATION, MOTTLED	LUNG - Not required by protocol
LUNG - CARDIAC LOBE, PIGMENTATION, MOTTLED	LUNG - Not required by protocol
LUNG - DIAPHRAGMATIC LOBE, PIGMENTATION, MOTTLED	LUNG - Not required by protocol
SPLEEN - PIGMENTATION, PALE	SPLEEN - Not required by protocol
LYMPH NODE,BRONCHIAL - PIGMENTATION, DARK	LYMPH NODE,BRONCHIAL - Not required by protocol
STOMACH - CARDIAC, FOCUS, 2X2 MM, MULTIPLE, RED	STOMACH - Not required by protocol
STOMACH - FUNDIC, FOCUS, 3X3 MM, MULTIPLE, RED	STOMACH - Not required by protocol
SMALL INTESTINE,DUODENUM - PIGMENTATION, MULTIPLE, DARK	SMALL INTESTINE,DUODENUM - Not required by protocol

PATHOLOGY ASSOCIATES
FOUR-WEEK ORAL (GAVAGE) TOXICITY STUDY OF
1-ALPHA-HYDROXYVITAMIN D5 IN BEAGLE DOGS
IIT RESEARCH INSTITUTE PROJECT NUMBER 1209 STUDY NUMBER 2

CORRELATION OF GROSS & MICRO

STUDY ID: 1209 SN2
SEX: FEMALE

STUDY NUMBER: 1209SN2
GROUP: 4: 45 ug/kg body weight

Animal ID: 1248
Animal Fate: Natural death

Days on Test: 6

Reference to Necropsy Record:	Related Histopathology:
LARGE INTESTINE,RECTUM - PIGMENTATION, MULTIPLE, DARK	LARGE INTESTINE,RECTUM - Not required by protocol

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19-JAN-2001

SECTION VI

QUALITY ASSURANCE STATEMENT